ASSESSMENT OF MATERNAL VITAMIN D STATUS IN GESTATIONAL DIABETES MELLITUS

Dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY,

CHENNAI – 600032

In partial fulfillment of the requirement for the degree of

Doctor of Medicine in Physiology (Branch V)

M.D. (PHYSIOLOGY)

APRIL 2016

DEPARTMENT OF PHYSIOLOGY

COIMBATORE MEDICAL COLLEGE

COIMBATORE – 14

CERTIFICATE

This dissertation titled **"ASSESSMENT OF MATERNAL VITAMIN D STATUS IN GESTATIONAL DIABETES MELLITUS "** is submitted to The Tamilnadu Dr.M.G.R Medical University, Chennai, in partial fulfillment of regulations for the award of M.D. Degree in Physiology in the examinations to be held during April 2016.

This dissertation is a record of fresh work done by the candidate **Dr. U.KALPANA RANI ,** during the course of the study (2013 - 2016).

This work was carried out by the candidate herself under my supervision.

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DECLARATION

I, **Dr. U.KALPANA RANI** solemnly declare that the dissertation titled **"ASSESSMENT OF MATERNAL VITAMIN D STATUS IN GESTATIONAL DIABETES MELLITUS"** was done by me at Coimbatore Medical College, during the period from July 2014 to June 2015. Under the guidance and supervision of **Dr.P.MURUGESAN,M.D.,** Professor, Department of Physiology, Coimbatore Medical College, Coimbatore. This dissertation is submitted to The Tamilnadu Dr .M.G.R. Medical University towards the partial fulfillment of the requirement for the award of M.D. Degree (Branch -V) in Physiology.

I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

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College : COIMBATORE MEDICAL COLLEGE

Dissertation Topic : ASSESSMENT OF MATERNAL VITAMIN D STATUS IN GESTATIONAL DIABETES MELLITUS

The Ethics Committee, Coimbatore Medical College has decided to inform that your Dissertation Proposal is accepted / ~~Not accepted~~ and you are permitted / ~~Not permitted~~ to proceed with the above Study.

DEAN
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15.7.2014

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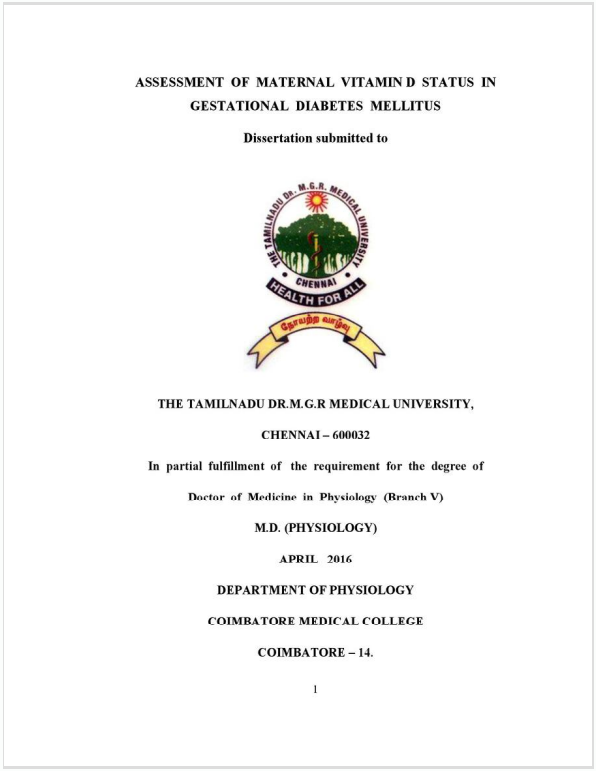

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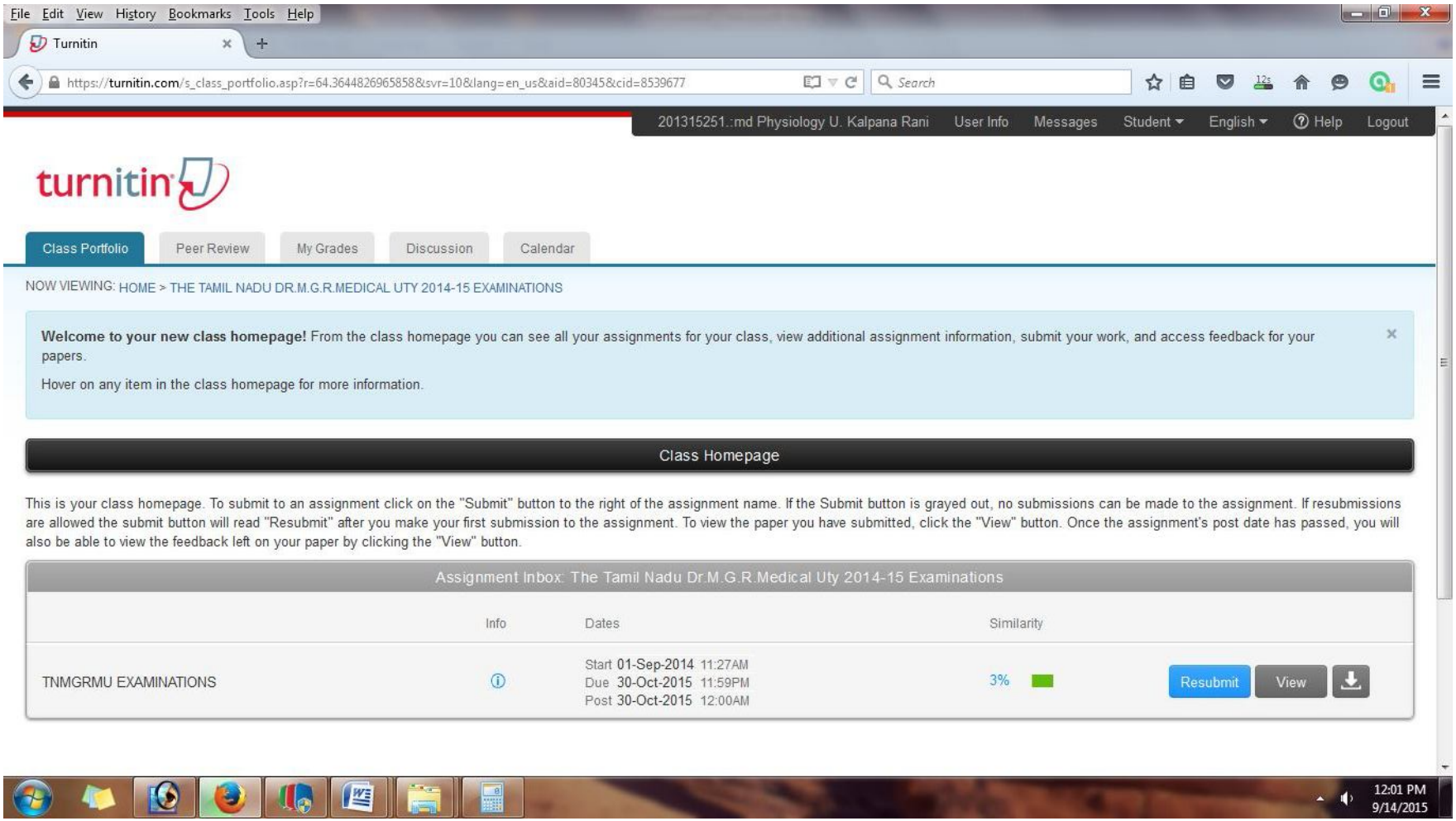
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ASSESSMENT OF MATERNAL VITAMIN D STATUS IN GESTATIONAL DIABETES MELLITUS

CONTENTS

ABBREVIATIONS USED IN THE STUDY

GDM	- Gestational Diabetes Mellitus
OGTT	- Oral Glucose Tolerance Test
25(OH)D	- 25-Hydroxyvitamin D
$1,25(OH)_2D$	- 1,25-dihydroxy vitamin D
VDD	- Vitamin D Deficiency
Vit D	- Vitamin D
OGCT	- Oral Glucose Challenge Test
WHO	- World Health Organisation
NGT	- Normal Glucose Tolerance
VDBP	- Vitamin D Binding Protein
VDR	- Vitamin D Receptor
RXR	- Retinoid X Receptor
PTH	- Parathyroid Hormone
TGF	- Transforming Growth Factor
TNF	- Tumour Necrosis Factor

PTHrP	- Parathyroidhormone –related peptide
ELISA	- Enzyme Linked Immuno sorbent Assay
LC-MS	- Liquid Chromatography –Tandem Mass Spectrometry
PPARγ	- Peroxisome Proliferator-Activated Receptor Gamma
PGC1 α	- PPAR Gamma Coactivator alpha
IRS -1	- Insulin Receptor Substrate -1
GLUT -4	- Glucose Transporter -4
BMI	- Body Mass Index
GOD- POD	- Glucose Oxidase – Peroxidase
CLIA	- Chemi Luminescence Immuno Assay
RLU	- Relative Luminosity Values

INTRODUCTION

INTRODUCTION

"*Pregnancy is a physiological process that invites a woman to yield to the unseen power behind all life where soul and spirit are stretched*". But, pregnancy has its own risks . We know, diabetes mellitus is a rapidly growing metabolic disarray in modern era, mainly due to insulin resistance, referred as diabesity[1]. Gestational Diabetes Mellitus (GDM) is one of the commonly encountered medical problem, in pregnancy[1, 2].

GDM is termed as glucose intolerance of unpredictable severity with first detection or onset in pregnancy[1]. The incidence of gestational diabetes mellitus varies from 1 to 14%[2]. Women who are detected to have GDM, have an increased possibility of acquiring diabetes later on. So, identification of GDM is a major health concern.

Pregnancy is a diabetogenic state as insulin requirements during pregnancy are increased[3]. All the changes in carbohydrate metabolism are mainly directed towards making more glucose available to the fetus[4]. As the pregnancy advances, insulin resistance increases as the placental hormones like human placental lactogen, prolactin and cortisol, which has anti-insulin effect also increases[3,4].

The pregnancy hormones enhances lipolysis, which leads to increased free fatty acids which in turn aggravates insulin resistance[4]. The pancreas is stimulated to produce more insulin and plasma glucose levels fall. Hence fasting hypoglycemia with postprandial hyperglycemia and hyperinsulinemia are the characteristics of normal pregnancy.[1]

Gestational diabetes mellitus develops, when pancreas despite the increased production of insulin cannot counter the insulin resistance caused by the pregnancy hormones. So the clinical expression of gestational diabetes occurs when the compensation is inadequate, usually during the latter half of pregnancy[4]. GDM usually develops in women with a poor pancreatic reserve and insulin resistance such as those with polycystic ovary syndrome or a family history of diabetes. It appears usually after 24 weeks of pregnancy[4].

The known risk factors which predisposes a pregnant women to an increased possibility of developing diabetes in pregnancy include[3] : maternal overweight and obesity (> 120% ideal body weight), race/ethnicity-Asian ethnic background, prior record of GDM, history of previous fetal death, previous large babies (>4 kg), macrosomic infant in present pregnancy, hydramnios in present pregnancy, family history of diabetes mellitus, and increasing maternal age[3.]

Vitamin D has been well recognized for its beneficial effect on bone health. Recent discovery is that, receptors for vitamin D are identified in many body tissues that influence the metabolism of blood glucose , like beta cells of pancreas, muscle, and placenta[5]. Reports have shown that, one of the preventable cause for GDM is vitamin D deficiency (VDD) [6].

Vitamin D is a secosteroid compound. The main source of vitamin D is exposure to sunlight[5]. Vitamin D is synthesized photochemically from 7- dehydrocholesterol present in basal layers of skin under the influence of sunlight[5]. Deficiency of vitamin D is more common in spite of abundant sunlight in India. The sources of vitamin D in diet are egg yolk, cod liver oil, fatty fish, fortified food products like fortified oranges, fortified milk, yogurt, cheese and cereals[5 - 7].

Cultural and social taboos has an impact on life style patterns such as clothing, lack of outdoor activities and working in air conditioned rooms reduce the exposure to sunlight and also vegetarian diet has low vitamin D concentrations[8]. In India, the prevalence of VDD is reported to be about 15 - 80%[9]. During pregnancy, due to active transplacental calcium transport to the developing fetus, there is an increased possibility of VDD[10].

Vitamin D deficiency is pandemic throughout the world, but it is the poorly diagnosed and poorly treated nutritional deficiency all over. It not only has its influence on skeletal tissues but also influences the non-skeletal tissues. Multiple tissues in our body like placenta, breast, lung, colon, prostate, bone, parathyroid, pancreas, immune system express vitamin D receptors[5].

VDD during pregnancy has a special importance as it has a chance to affect both the mother and fetus[9]. During pregnancy, deficient levels of vitamin D is linked to adverse effects on mother like preeclampsia, obesity, insulin insensitivity, and risk of GDM, bacterial vaginosis, and increased rates of cesarean delivery [9,11]. Deficiency of vitamin D on newborns leads to low birth weight (LBW), rickets, diabetes mellitus , hypocalcemia in neonates and bronchial asthma [9].

The active metabolite of vitamin D, 1,25-dihydroxy vitamin D is found to regulate the secretion of beta cells by complexing to the vitamin D receptors present in the beta cell and also helps in maintaining the equilibrium between the intracellular and extracellular calcium stores[5]. It also enhances insulin sensitivity and response by inducing the expression of insulin receptors [12].

Newer Studies have proposed that VDD is related to reduced insulin release and also insulin resistance which is managed by administering vitamin D [13,14]. Vitamin D has a major influence in the development of type2 diabetes by influencing the sensitivity of insulin or function of beta cells[15].

Accumulating evidences associate VDD with impaired glucose homeostasis. Epidemiological reports have revealed that women with VDD, had a higher risk of GDM . The vitamin D status in the body is assessed by estimating the 25-hydroxyvitaminD levels (25(OH)D)[8].

Yazd from Iran, studied the glucose tolerance in 126 healthy subjects and reported a direct correlation between 25-hydroxyvitaminD concentrations and sensitivity of insulin[16].

Maghbooli et al., found that 25-hydroxyvitaminD concentrations estimated during second trimester of pregnancy were found lower in women with Gestational diabetes compared to women with normal pregnancy[17].

Keeping all these in mind, in this study, an endeavor has been made to assess the maternal vitamin D levels in GDM and to find any relationship between deficiency of vitamin D and GDM, so that it may be possible for the treating physicians to provide essential care and caution in the identification and management of VDD during pregnancy.

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

AIM :

- To assess the serum vitamin D status in women with GDM.

OBJECTIVES :

- To estimate the serum total 25-hydroxyvitamin D levels in women diagnosed having GDM.
- To estimate the levels of serum total 25-hydroxyvitamin D in normal pregnant women.
- To compare the concentrations of total 25-hydroxyvitamin D between women diagnosed with GDM and normal pregnant women.
- To find correlation between the blood sugar levels and total 25-hydroxyvitamin D concentrations in GDM.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Gestational Diabetes Mellitus is intolerance of glucose metabolism of unpredictable severity with first identification or onset in pregnancy[1]. The incidence of GDM is globally increasing with no exception to India and varies between 1 to 14%[2]. GDM is the major contributor for poor maternal and fetal outcome. It is also responsible for the future health risks in mother as well as offspring.

Antenatal women with glucose intolerance can be classified into pre-gestational and gestational diabetes mellitus[3]. Pregnancy is a diabetogenic state as insulin requirements during pregnancy are increased[3]. Women with gestational diabetes are individuals with a genetic or metabolic predisposition towards diabetes and are incapable of adequately compensating for the diabetogenic effects of pregnancy. The most important reason why pregnancy increases the diabetic tendency of asymptomatic women is due to the progressive increase in insulin resistance[18].

FUEL METABOLISM IN PREGNANCY

During pregnancy, there is an enhanced insulin action in first trimester and more diabetogenic stress in second trimester[19]. During early weeks of pregnancy, there occurs beta cell hyperplasia due to increasing concentrations of estrogen and progesterone resulting in hyperinsulinemia. Insulin which is an anabolic hormone, favours glycogen storage in tissues, inhibits gluconeogenesis and favours peripheral utilization of glucose[19].

Insulin requirements during pregnancy starts from third month and continues till term. In second trimester, synthesis of the placental hormones like human placental lactogen (HPL), estrogen, prolactin, and cortisol increases which are responsible for the insulin resistance and glucose intolerance[19]. Then there occurs production of enzymes like placental insulinase by the placenta that increases the degradation of insulin[3]. The hyperglycemic state which occurs due to the above reasons is to provide a continuous source of glucose to the fetus[1]. As a result the metabolic changes that occur under the influence of insulin and the anti-insulin hormones facilitate anabolism during feeding and catabolism during fasting[19].

SUBSTRATE DEFICIENCY SYNDROME

As the pregnancy advances, there is a constant utilization of glucose by the growing fetus, which keeps a low plasma fasting glucose level. The fasting hypoglycemia is also attributed to a fall in circulating aminoacids like alanine which is needed for gluconeogenesis. This situation is called 'substrate deficiency syndrome'. The growing fetus removes glucose by facilitated diffusion and alanine by active transport from the maternal circulation[19]. As a result of the normal physiological changes during pregnancy, the mean fasting blood sugar value is 65±9 mg/dl and the mean non-fasting blood sugar level is 80±10 mg/dl. The post-prandial blood sugar levels never exceed 140 mg/dl in normal pregnancy[18].

ACCELERATED STARVATION STATE IN PREGNANCY

The plasma levels of placental lactogen increase with gestation. The placental lactogen has growth hormone like action that causes increased lipolysis with liberation of free fatty acids (Freinkel, 1980). The increased circulating free fatty acid concentration leads to increased peripheral insulin resistance[1]. Pregnancy is characterized by fasting hypoglycemia due to increased plasma concentrations of free fatty acids, triglycerides, and cholesterol levels[19].

Freinkel and colleagues (1985) have named this pregnancy induced switch in fuels from glucose to lipids as 'accelerated starvation'[1]. These metabolic changes occur as early as 18 hours of food deprivation during pregnancy compared to that occurs 72 hours after food deprivation in a non-pregnant state[19].

In a nutshell , accelerated starvation in the fasting and facilitated anabolism in postprandial state influences the maternal fuel adaptations during pregnancy[19]. But as the pregnancy advances, insulin resistance also increases as the placental hormones increases which necessitates a compensatory increased insulin secretion. So the clinical expression of GDM occurs when this compensation is inadequate, usually during the latter half of pregnancy[19].

PATHOPHYSIOLOGY OF GLUCOSE INTOLERANCE DURING PREGNANCY

- **GESTATIONAL FACTORS**

1. **Insulin resistance** : There is increased synthesis of counter hormones like human placental lactogen (HPL), cortisol, prolactin, estrogen and progesterone leading to insulin resistance. Normally, there is a 30% increase in endogeneous insulin synthesis during pregnancy. Studies have shown that when women with normal glucose tolerance were given HPL, and challenged with oral glucose load, these women

showed failure to increase plasma insulin levels. Those women who are unable to increase their insulin secretion to compete the insulin resistance develop gestational diabetes mellitus[19].

2. **Secretion of insulin** : In women with GDM, the early release of insulin is sluggish during the oral glucose challenge test. The ratio between insulin and glucose is reduced[19].

GDM - A FORM OF TYPE 2 DIABETES

GDM is a type of NIDDM discovered in pregnancy. Catalano et al., (1999) compared prospective changes in insulin response and sensitivity as well as synthesis of glucose between women with GDM and normal pregnancy. Women with GDM showed abnormal carbohydrate metabolism that are characteristic of type 2 diabetes mellitus[1].

The term GDM is used because it helps in providing increased surveillance and also stimulates the women to seek care for testing plasma glucose postpartum. More than 50% of the women with GDM develop overt diabetes in next 20 years. There is increasing evidence for future complications that include obesity, Metabolic syndrome and diabetes mellitus in their offspring[1].

CLASSIFICATION OF DIABETES MELLITUS IN PREGNANCY

It is important to describe the evolution of diabetes classification during pregnancy over the past 20 years (because many older terms continue to be used). In olden days , classification of diabetes was based on National Diabetes Data Group (NDDG) [1,18].

NATIONAL DIABETES DATA GROUP [1,18].

I. Type 1- Immune-mediated and/or idiopathic beta cell damage which leads to absolute deficiency of insulin.

II. Type 2- Insulin resistance with relative deficiency of insulin.

III. Others - Genetic defects affecting beta cell action and/or insulin action, exocrine pancreatic diseases or endocrinopathies like Cushing syndrome, chemical or drug induced like glucocorticoids or infections like congenital rubella, cytomegalovirus.

IV. Gestational Diabetes Mellitus [1, 18]

WHITE CLASSIFICATION OF DIABETES DURING PREGNANCY[1]

In 1978, Priscilla White classified Diabetes in pregnancy.

- Type A - Chemical diabetes
- Type B - Maturity onset (above twenty years) with period of diabetes of less than 10 years with no complication.
- Type C - Onset -10 to 19 years of age, with period of diabetes -10 to 19 years with no complication.
- Type D - Age less than 10 years - diabetic duration >20 years with retinopathy (benign)
- Type F – Any age - any duration of diabetes and Nephropathy
- Type R – Any age - any period of diabetes with proliferative retinopathy.
- Type H - Any age with any duration with cardiomyopathy.

In 1986, the American College of obstetricians and Gynaecologists (ACOG) recommended a classification for diabetes in pregnancy[1]. This is same as White's classification except the chemical diabetes was replaced by:

- Class A_1 –GDM with fasting plasma glucose (FPG) level <105 mg%, 2hr post-prandial (PP) levels < 120 mg%.
- Class A_2 –GDM with FPG >105mg% , 2 hr PP plasma glucose more than 120 mg%[1].

IMMUNE RESPONSE AND ROLE OF PLACENTA IN GDM

Recent evidences have shown that there is a decreased activity of pro-inflammatory T-helper cells (Th1) and adiponectin with enhanced action of leptin and inflammatory cytokines like IL-6, TNF-alpha in women with GDM[20].

Studies have shown that placenta expresses repertoire of inflammatory cytokines which become over expressed in a diabetic environment[20]. TNF-alpha may participate in pregnancy - induced insulin resistance. All the above events show that inflammation is linked with changes in glucose metabolism leading to insulin insensitivity in pregnant women[20].

RISK FACTORS OF GDM

The known risk factors which predispose a pregnant women to an increased possibility of developing diabetes in pregnancy include [3] :

- Maternal overweight and obesity (> 120% ideal body weight)
- Race/ethnicity - Asian ethnic background
- Previous history of GDM
- Previous large babies (>4 kg)
- Macrosomic infant and/or hydramnios in present pregnancy
- First degree relatives with DM
- Increasing maternal age[3].

Recent discovery proves that receptors for vitamin D are located in muscle and beta cells of pancreas which are concerned with glucose homeostasis [19]. Studies have revealed that GDM may occur from insulin resistance induced by pregnancy and decreased compensatory insulin release[21]. Newer studies have suggested that immune responses in GDM are due to deficiency of vitamin D[20]. Studies also have shown that vitamin D deficiency has been found to be one of the risk factors for GDM [21].

EFFECT OF DIABETES ON PREGNANCY

MATERNAL[3]:

- Higher risk of abortions
- Maternal urinary tract infections
- Monilial vulvovaginitis
- Preterm labour
- Pregnancy induced hypertension
- Hydramnios.

FETAL EFFECTS[3]:

- Neural tube defects like spina bifida, anencephaly, Caudal regression syndrome
- Fetal macrosomia
- Cardiac anomalies like transposition of great vessels and septal defects
- Renal anomalies like agenesis, duplex ureter and cystic kidney
- Unexplained fetal death

GDM – A LIKELY CAUSE FOR TYPE 2 DIABETES IN FUTURE [1]

There is a 50% likelihood of women with GDM developing overt diabetes within 20 years. Metzger and associates reported that when fasting blood glucose levels during pregnancy were between 105 to 130 mg/dl, 43% of women with GDM were found to develop overt diabetes and when fasting blood glucose exceeds 130mg/dl during pregnancy, 86% were found to develop overt diabetes. Dacus and coworkers reported that, insulin therapy during pregnancy, especially before 24 weeks, is a powerful predictor of persistent diabetes[1]. Women diagnosed to have GDM are more prone for cardiovascular problems associated with hypertension, dyslipidemia and abdominal obesity. Holmes and colleagues in 2003 reported that the recurrence of GDM in subsequent pregnancies was 40%[1].

SCREENING FOR GESTATIONAL DIABETES MELLITUS

Despite research for more than 40 years , there is no consensus about the optimal approach to screening for the GDM (ACOG, 2001)[1]. Women who are more prone for developing GDM need to be identified and managed to get a favourable pregnancy outcome[3]. An Asian ethnic background is a major contributor and hence, screening for GDM should be offered to all Indian pregnant women[3].

SCREENING METHODS FOR GDM

O'Sullivan test or Oral Glucose Challenge Test (OGCT)

This test is performed between 24 and 28 weeks of pregnancy, as the insulin demand increases after 24 weeks of gestation due to the insulin antagonistic effect by the increasing pregnancy hormones[3]. The plasma glucose is measured one hour after a 50gm oral glucose load irrespective of the last meal. A value of $\geq$140 mg/dl helps to identify 80% of women with GDM[3].

Glucose Tolerance Test (GTT)

when the screening is positive or when the antenatal women is unwilling to do a two-step procedure (screening and then GTT)[3] , this test is employed.

Procedure: The Pregnant woman is instructed to take a normal diet the previous day. After an overnight fasting of 8-14 hours, a fasting blood glucose sample is taken. Then the pregnant women are given 100g of glucose in 200 ml of water orally. Venous blood samples are collected at the end of 1 hour , 2 hours and 3 hours. The cut-off values for diagnosing gestational diabetes as given by Carpenter and Coustan and (NDDG) are given below. Any results with two or more abnormal values are diagnostic of GDM[3].

RESULTS OF GTT

Time	**Carpenter & Coustan**	**NDDG**
FBS	95 mg/dl	105 mg/dl
1 hr	180 mg/dl	190 mg/dl
2 hrs	155 mg/dl	165 mg/dl
3 hrs	140 mg/dl	145 mg/dl

Disadvantages of GTT:

1. Glycemic cut off is not concerned with fetal outcome .
2. Too many blood samples have to be given
3. Pregnant women have to attend clinic twice: a) screening b) diagnosis[19, 22].

The World Health Organisation (WHO) criteria for screening diabetes mellitus -75 gm OGTT

This is a 75 gm, 2 hour oral glucose tolerance test[22] . This test is done to standardize the diagnosis of GDM. A cut-off value of ≥140mg/dl of plasma glucose after 2 hours of 75 gm oral glucose. The test is done irrespective of last meal. Nowadays, 75 gm GTT is replacing the 100 gm GTT[19,22].

Advantages of 75 Gram OGTT

- Best recommended for its easy adaptability and sensitivity
- Fasting is not necessary in a pregnant women
- Routine activities of the women are not affected
- Helps in both screening and diagnosing GDM[6].

RESULTS OF 75 gm OGTT (WHO criteria)[19]

2 hours blood Glucose	**PREGNANT STATE**	**NON-PREGNANT STATE**
2 hour ≥ 200 mg%	Overt Diabetes	Overt Diabetes
2 hour ≥ 140 mg% and ≤ 199mg%	Gestational diabetes mellitus	Impaired Glucose Tolerance
2 hour ≥ 120 mg% and ≤ 139 mg%	Gestational Glucose Intolerance	----------
2 hour< 120 mg%	Normal glucose tolerance	Normal glucose tolerance

SIGNIFICANCE OF THE CUT-OFF POINT

The increasing glucose intolerance in the pregnant women is associated with complications to both mother and the fetus. The birth weight of the fetus and serum C peptide levels of more than 90th percentile happen at the cut-off level of 2 hr plasma glucose >140 mg%. The possibility of type2 DM in children increases in women with third trimester plasma glucose levels of 120 to 139 mg/dl. So this cut-off point has its significance[22].

WEEKS OF PREGNANCY DURING WHICH GDM IS SCREENED

In the fetus, insulin secretion starts by the 16th week of pregnancy in response to increased maternal glucose levels. However, insulin is detected in fetus at 9th week itself. The screening for GDM is recommended at 24 to 28 weeks of gestation usually. It is better to screen for GDM in I trimester itself to avoid missing women with pre-gestational diabetes. Pregnant woman with normal glucose tolerance (NGT) during I trimester are adviced to undergo GDM screening in the second trimester and lastly around 32nd – 35th week[22].

EDWARD MELLANBY

ADOLF OTTO REINHOLD WINDAUS

VITAMIN D

History

There were reports of symptoms of rickets from history as early as 2nd century AD. In 1919, Edward Mellanby found that a factor was present in cod liver oil which prevented rickets in dogs when the dogs were given cod liver oil. In 1922, Elmer McCollum who identified vitamin A , isolated a substance, from cod liver oil from which vitamin A was removed. McCollum found that the isolated substance, cured rickets in dogs, and named that fat soluble substance as vitamin D[23].

Early in 20th century, studies reported that children with rickets were cured after exposing them to sunlight . Goldmann and Soames identified that vitamin D is synthesized on irradiation of skin, as well as irradiated rat liver could cure rickets. Similar observations were given by Weinstock. Hess along with Unger identified that sunlight can alleviate rickets . Huldshinsky was the one who argued that even artificial light can be employed to cure rickets and improve the calcium deposition[23]. Adolf Windaus who was a German chemist discovered the structure of vitamin D_3. Windaus was awarded the nobel prize for the same in chemistry in 1928[6,23]. The work was also contributed by Hess and Rosenheim[23].

VITAMIN D – FORMS [24]

VitaminD_2 (ergocalciferol) and vitaminD_3 (cholecalciferol) are the two forms of vitaminD. A 28-carbon molecule, ergocalciferol, is synthesized by Ultraviolet irradiation of yeast and the plant ergosterol[7]. Cholecalciferol is produced from 7-dehydrocholesterol present in the epidermis of human and animal skin after sun exposure. The storage, transport, metabolism and biological potencies of these two forms are equivalent and effectively stimulated by the hydroxylases in humans[24].

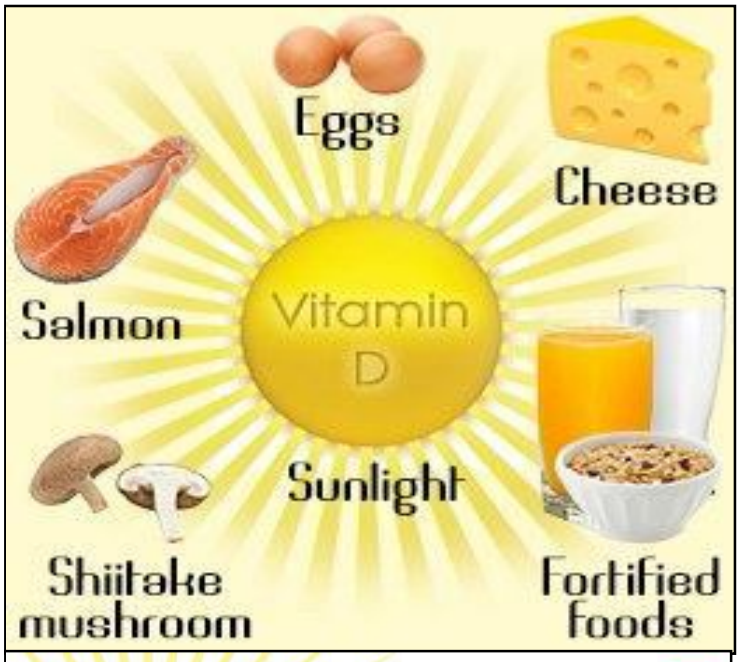
VITAMIN D – SOURCES

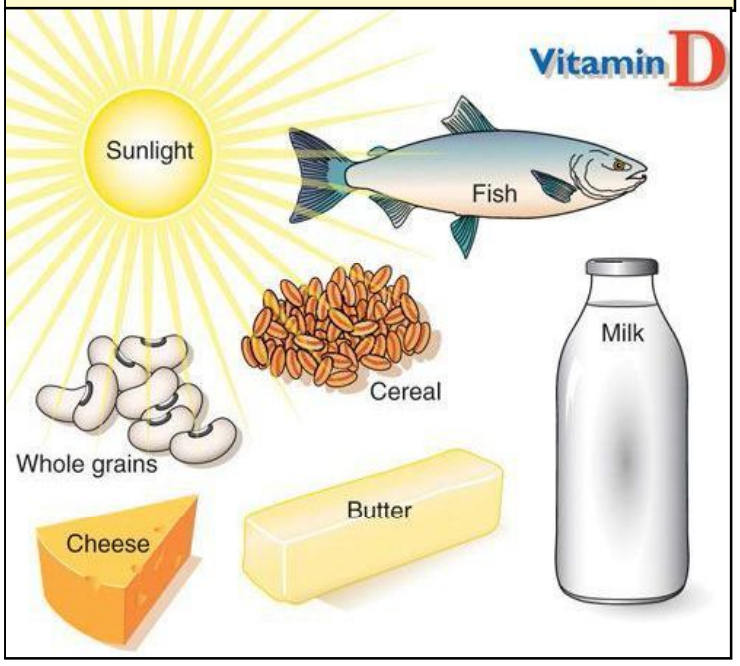
Sun exposure – a major source

Exposure to ultraviolet (UV) radiations of sun is the major source of vitamin D. When exposed to UV irradiations from sunlight, 7-dehydro cholesterol, which is the cutaneous precursor of vitamin D, undergoes photochemical changes leading to formation of vitamin D[24]. The photochemical synthesis of vitamin D in the skin depends on the amount of the UVB photons that strike the basal epidermis. Glass, clothes, sunscreen, and skin pigment the UVB light and blunt vitamin D synthesis[6].

India receives adequate sunlight throughout the year as it is a tropical country. The wavelength of the ultraviolet radiation B (UVB) required for vitamin D synthesis is 290 -315 nm[6]. Exposure of skin especially, arms and legs to sunlight , without application of any

SOURCES OF VITAMIN D





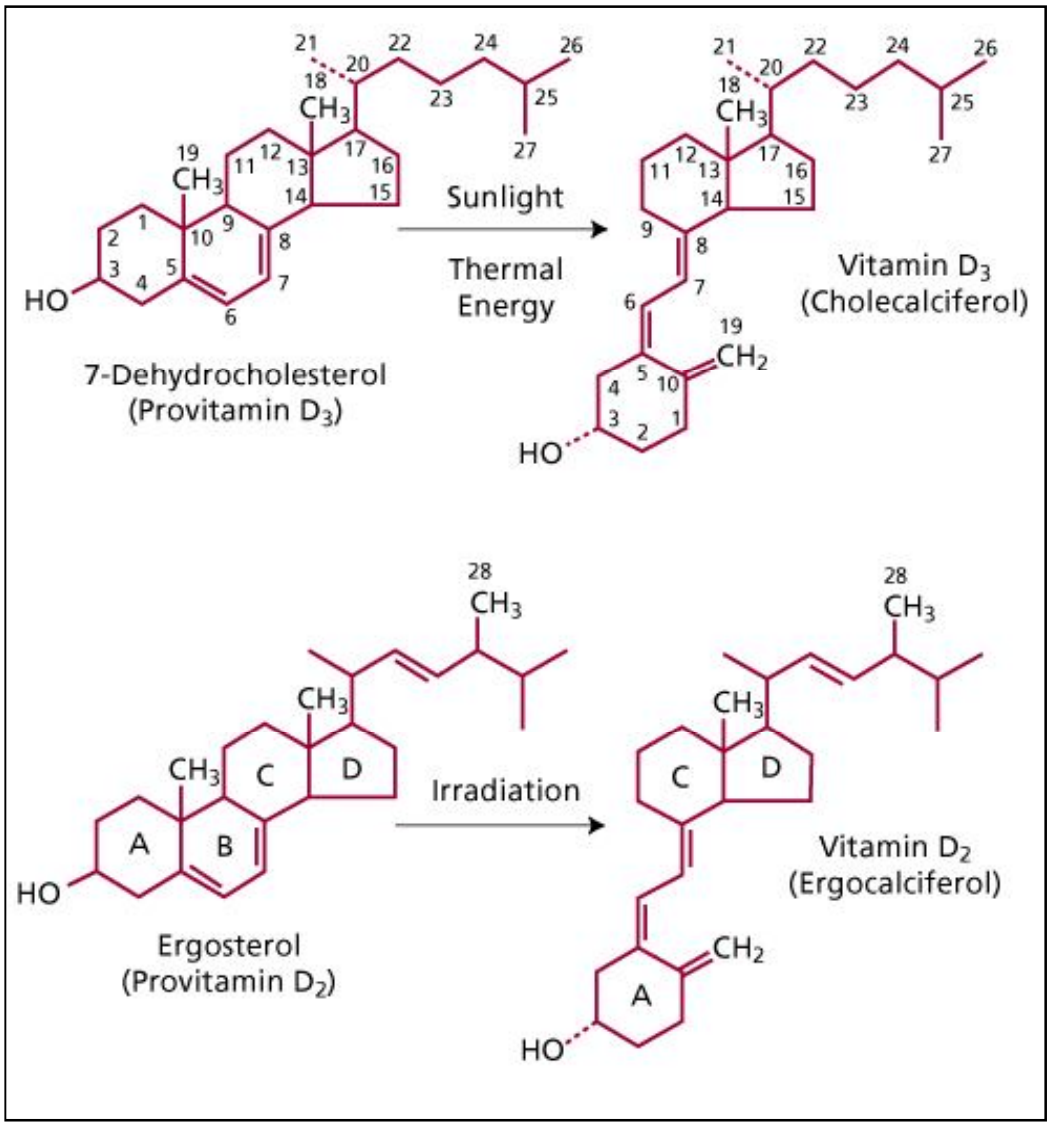
sunscreen for thirty minutes, preferably between 10 am to 2 pm daily is adequate for vitamin D synthesis as maximum UVB rays are transmitted during this time[25]. Latitude, seasonal changes, and the day's time factor influence the intensity of solar radiation and affects the synthesis of vitamin D. There is a risk of VDD during winter and spring[6].

Dietary Sources

Vitamin D_3 is found in cod liver oil, egg yolk, and fish[6]. Our human diet is poor in vitamin D except fatty fish. The wild salmon fish provides 600 -1000 IU. Fishes like Mackeral, sardine, and tuna fish provides 300 IU of vitamin D. One teaspoon of cod liveroil supplies about 600 to 1000 IU of vitamin D. Shiitake mushrooms offer about 1600 IU of vitamin D. The other dietary food sources are fortified food products like fortified oranges, fortified milk, yogurt, cheese and cereals[7]. Vitamin D is not present in significant amounts in meat, diary products and meat which is not fortified[7].

Elderly people and institutionalized individuals obtain most of their vitamin D from dietary sources. But still the dietary product contribution to the circulating levels of vitamin D is low compared to that produced from exposure to sunlight[7]. Human and cow's milk are poor sources of vitamin D, providing 15 to 40 IU/L[6].

PHOTOSYNTHESIS OF VITAMIN D



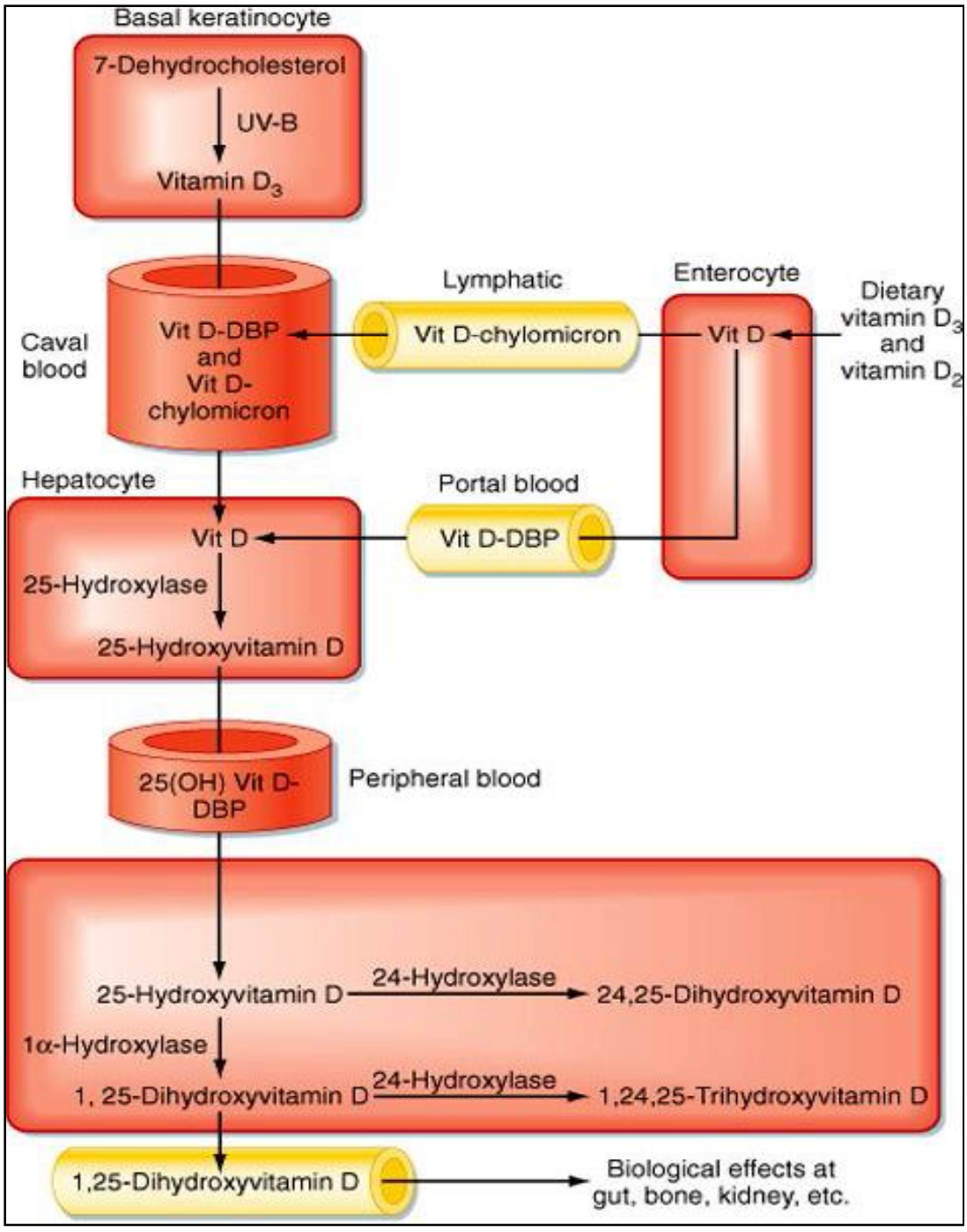
PHOTOSYNTHESIS OF VITAMIN D

The 7-dehydrocholesterol or provitaminD_3 is present abundant in keratinocytes of the basal or spinous epidermis layers of the skin. Under the influence of UVB radiations, it undergoes photochemical cleavage of the carbons 9 and 10 of the steroid ring and converted into pre-vitamin D_3[6].

The synthesis of previtamin D_3 is a non-enzymatic photo chemical reaction. The previtamin D_3 is thermally labile and undergoes a temperature dependent molecular rearrangement over a period of 48 hours that results in the formation of vitamin D_3. Moreover, previtamin D_3 can isomerise to two biologically inert products, luminosterol and tachysterol[24]. This alternative photo isomerisation prevents production of excessive amounts of vitamin D with prolonged sun exposure. So vitamin D intoxication does not occur on excessive sun exposure[24].

Both the forms of vitamin D are added into the chylomicrons and taken into the venous system with the help of lymphatics. The generic term, vitamin D is used commonly for both forms. Fat cells are the storage place for vitamin D from where it is released on need [26]. Then the vitamin binds to vitamin D binding protein and hydroxylated in liver[6].

METABOLISM OF VITAMIN D



METABOLISM OF VITAMIN D IN LIVER

In liver, 25-hydroxylase is the cytochrome P-450 like enzyme that converts vitamin D into 25- hydroxyvitaminD . This hydroxylation takes place in the mitochondria of liver cells[26,27]. The cofactors required for this hydroxylation are magnesium, NADPH, and molecular oxygen[26]. The 25-hydroxylation of vitamin D is not strictly regulated.

The 25- hydroxyvitamin D levels are employed as a marker for nutritional vitaminD status by the clinicians because this forms the substrate for renal and non- renal production of 1,25 $(OH)_2D$. The half life of 25-hydroxyvitaminD is longer about 2 - 3 weeks with increased concentrations in circulation. This form of vitamin D is biologically inactive[24].

METABOLISM IN KIDNEYS

In the kidneys, the conversion of biologically inactive 25-hydroxyvitaminD into biologically active 1,25-dihydroxyvitaminD (calcitriol) by an enzyme 25(OH)D 1alpha-hydroxylase[24]. The half-life of calcitriol is about 6 to 8 hours. This hydroxylation at 1 position takes place in the proximal convoluted tubules of kidney [27,28]. This reaction is a complex three component mono oxygenase reaction requiring magnesium, NADPH, molecular oxygen as cofactors. In addition to these cofactors, three more enzymes are required. They are ferrodoxin,

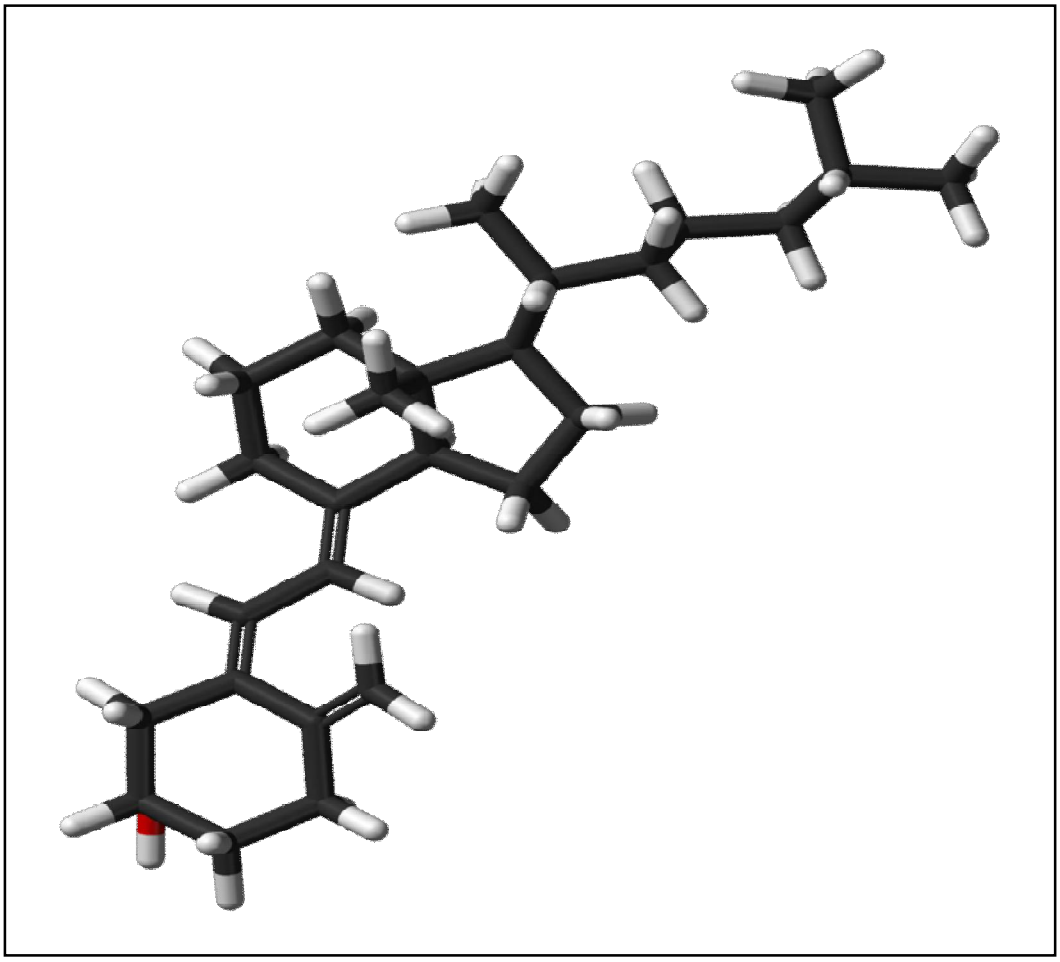
ferrodoxin reductase, and cytochrome P450. Calcitriol is the potent metabolite of vitamin D . Renal hydroxylation at C1 position is the most significant reaction , but similar hydroxylation also occurs in the placenta and bone[28].

Unlike, 25-hydroxylase, the 1-alpha –hydroxylase is strictly regulated. Parathormone, and hypophosphatemia are the two important stimulators of this microsomal enzyme. But calcium and calcitriol inhibit this enzyme[24,27]. Multiple tissues in our body like placenta, breast, lung, colon, prostate, bone, parathyroid, pancreas, immune system express vitamin D receptors and 1-alpha hydroxylase activity. These tissues have the ability to convert 25-hydroxy vitamin D into calcitriol[23].

Many tissues like kidney, cartilage, and intestine contain the enzyme 24-hydroxylase which hydroxylates 25-hydroxyvitaminD and calcitriol into 24,25-dihydroxyvitaminD and 1,24,25$(OH)_3$D respectively[24]. Calcitriol stimulates the activity of 24-hydroxylase and induces its own metabolism. The 24 -hydroxylated vitamin D metabolites are not found to have any major biological role.

Calcitriol is metabolized to multiple inactive products by 23 or 26 hydroxylation and side chain cleavage and oxidation. The side chain cleavage of calcitriol that leads to the formation of calcitroic acid occurs in liver and intestine[24].

STRUCTURE OF VITAMIN D



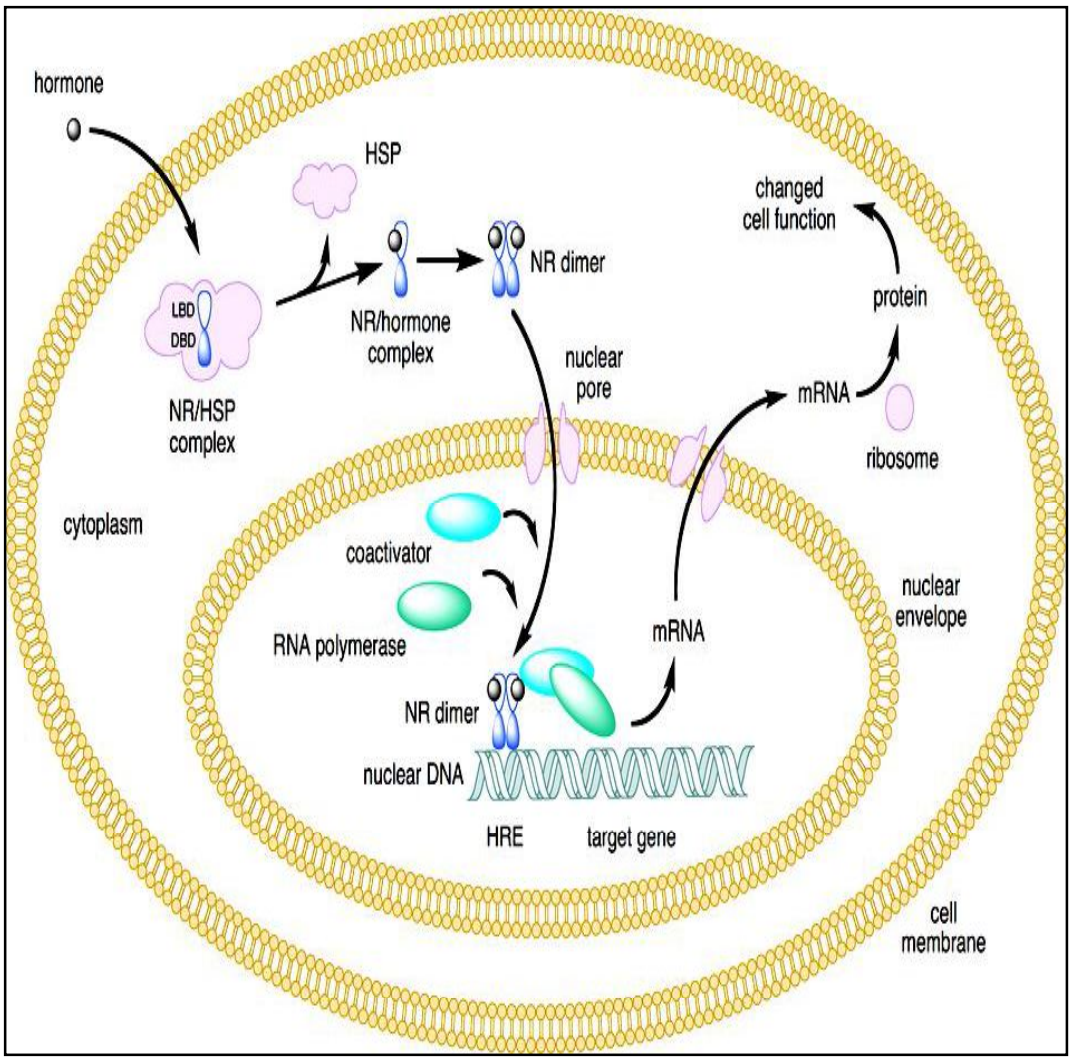
Vitamin D is esterified in the liver and then gets excreted mainly in the bile but some of its forms like calcitroic acid are excreted via urine[6]. Some of these metabolites are deconjugated in the intestine and reabsorbed into enterohepatic circulation [6].

VITAMIN D - AS A PROHORMONE[28]

Vitamin D cannot be considered as a true vitamin but it acts as a pro-hormone. The following features are consistent with the hormonal nature:

1. Structurally , vitamin D has cyclo- pentano perhydro phenanthrene ring like steroid hormone[28].
2. It is synthesized in the skin by UV irradiation from its precursor Pro-vitamin D3.
3. Vitamin D3 is inactive and only a storage form and conversion into the active form calcitriol occur in the kidneys.
4. It is transported in blood to distant sites in the body and activated by a regulated enzyme.
5. Like hormones, the active forms are subject to feed back inhibition.
6. It binds to specific receptors in target tissues like intestine, bone and kidneys to carry out its functions[29].
7. Calcitriol resembles the steroid hormone in mode of action , that it acts on nuclear receptors[28].

VITAMIN D - RECEPTOR BINDING & PROTEIN TRANSCRIPTION



VITAMIN D BINDING PROTEIN (VDBP)

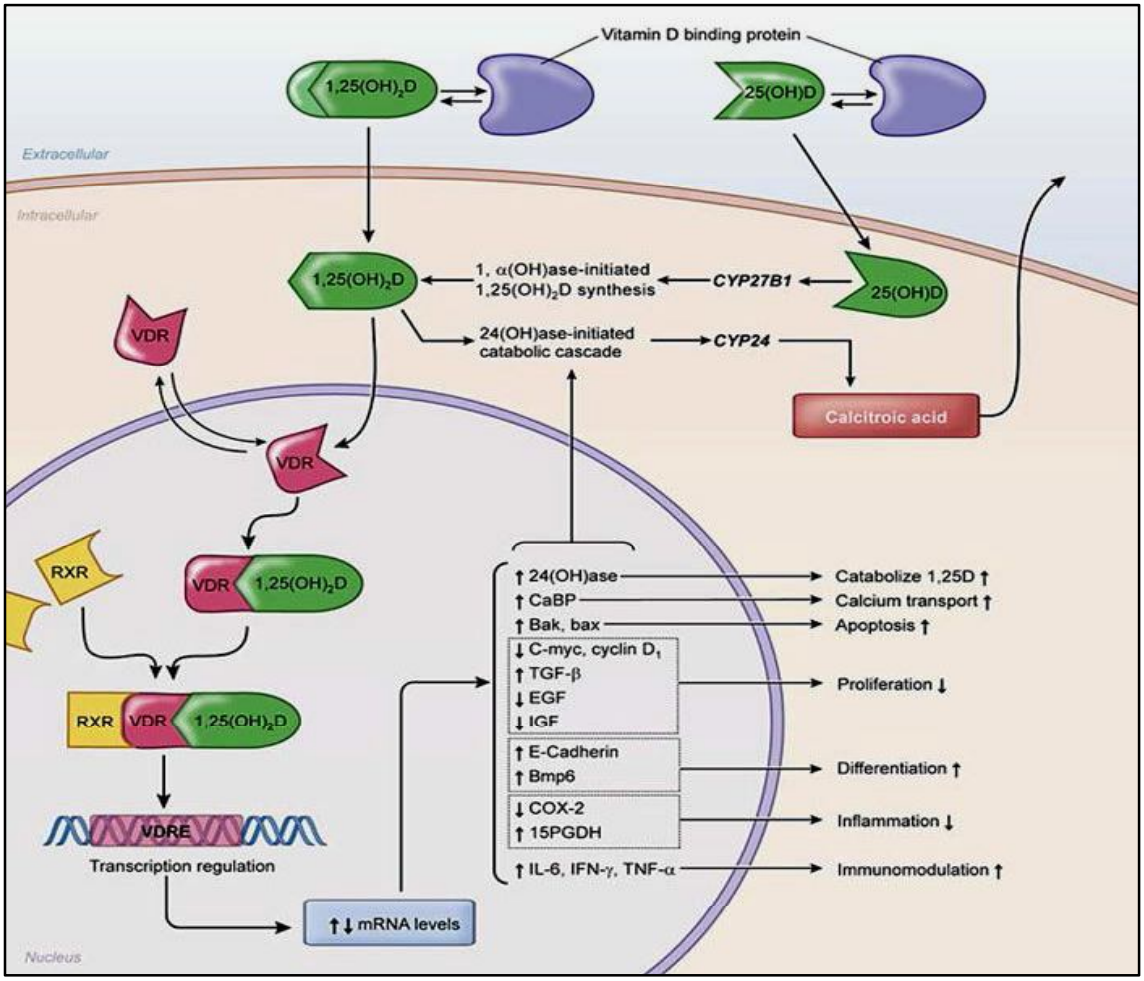
Vitamin D that enters the circulation is bound primarily to VDBP, but a fraction of vitamin D circulates bound to albumin[24]. The VDBP in human is a 52 –Kd alpha - globulin containing 458 aminoacids. The VDBP is produced from the liver. It was detected in 1959 as a group specific component or Gc-globulin[24]. The protein shows more binding capacity to 25-hydroxyvitamin D compared to calcitriol. About, 88% of 25(OH)D circulates bound to VDBP, 0.03% circulates freely, and the rest circulates bound to albumin[24]. The role of VDBP is to maintain a vitamin D reservoir in the serum and to modulate the activity of metabolites of vitamin D[24].

The function of VDBP in the endocrine system is assumed to reflect the 'free hormone' hypothesis. This hypothesis states that the free unbound vitamin D is responsible for the biological activity rather than the protein-bound fraction[6]. The plasma concentration of VDBP is increased by estrogen in most animal models. At the end of pregnancy, the concentration of VDBP gets doubled[6].

RECEPTORS OF VITAMIN D

Calcitriol binds with the Vitamin D receptor (VDR) in the nucleus, followed by DNA transcription into RNA for exerting the biological functions[24].

MECHANISM OF ACTION OF VITAMIN D



The human VDR gene, is located on chromosome12[6]. The VDR resemble the retinoic acid, triiodothyronine, and Retinoid-X Receptors (RXRs). The affinity of the receptors to calcitriol is three times greater than for other vitamin D metabolites. Though the levels of 25-hydroxyvitaminD in the serum, are three times greater than calcitriol, its affinity to the receptor is less than calcitriol. Under normal circumstances, 25(OH)D plays no much role in calcium homeostasis[24].

GENOMIC ACTIONS

Calcitriol binds to VDR which causes conformational changes in VDR followed by heterodimerization with unliganded RXR. It binds to vitamin D response elements (VDRE) which is present in the genes targeted for vitaminD and followed by the release of co-repressors and recruitment of co-activators. These co-activators control gene transcription by linking the receptor complex to the basal transcription apparatus[6].

NON-GENOMIC ACTIONS

Calcitriol has some biological effects which occur too rapidly through non-genomic actions[7]. These actions are mediated by a membrane receptor for $1,25(OH)_2 D$[6]. The non-genomic actions include a rapid increase in intracellular calcium, activation of phosphokinase C, and opening of calcium or chloride channels within minutes of exposure to calcitriol[24].

The vitamin D receptors are expressed in many tissues and has shown to regulate cellular differentiation and function in many types of cells and also intestinal calcium uptake[24].

REGULATION OF SYNTHESIS OF CALCITRIOL

The following factors regulate the synthesis of calcitriol :

1. **Plasma calcium** levels regulates calcitriol synthesis by a feed back mechanism indirectly through PTH. When serum calcium concentration increases, the concentration of PTH decreases which inturn increases the concentration of $1,25(OH)_2D$ and vice versa[30].
2. **Plasma phosphate** level regulates the synthesis of calcitriol by a feedback mechanism acting on the enzyme 1, alpha- hydroxylase directly. Decreased serum phosphate levels stimulate the activity of 1- alpha hydroxylase, that lead to an increase in calcitriol and vice versa[30].
3. **Calcitriol** shows direct negative feed back effect on its own formation by inhibition of 1- alpha hydroxylase activity and a positive feedback effect on the formation of $24,25(OH)_2D$ by stimulation of 24-hydroxylase[30].
4. **Other factors**[30]
    - Prolactin, Calcitonin and Growth hormone stimulate calcitriol synthesis

- Estrogen enhances the secretion of VDBP and increases the total circulatory $1,25(OH)_2D$
- Metabolic acidosis decrease the synthesis
- Hyperthyroidism decreases the circulating calcitriol levels .

VITAMIN D - CLASSICAL ACTIONS

Calcitriol exerts its classical action by acting at three different sites : intestine, bone, and kidneys to regulate the plasma calcium and phosphate levels [30].

ACTION ON INTESTINE

Under normal conditions, calcium ingestion is about 700 - 900 mg daily. About 30% to 35% of this calcium is absorbed. The important action of calcitriol is to help calcium absorption from the intestine[24]. The most extensively studied mechanism is the transcellular route. This involve three steps : Entry of calcium into the enterocyte, transport across the cell, and extrution across the basolateral membrane[24].

Vitamin D regulates the following levels of actions:

a. Increases calcium permeability at brush borders by causing changes in the membrane phospholipids. TRPV5 and TRPV6 are the calcium transport channels expressed in duodenum and jejunum. TRPV6 is for calcium absorption in intestine[24].

b. Induces the synthesis of calcium binding proteins, calbindin-D9K. These proteins help in carrying the calcium across the intestinal cell and play a role in buffering free intracellular calcium concentration during calcium absorption. The rate of absorption of calcium across the duodenum is proportional to the cell content of calbindin[30].

c. Promotes the entry of calcium from the cell cytoplasm into subcellular organelles , mainly mitochondria.

d. Stimulates the synthesis of calcium dependant ATPase which helps in pumping the calcium out of the cell. The affinity of pump for calcium is 2.5 times that of calbindin[24].

ACTION ON BONE

Calcitriol increases resorption of bone as well as mineralization of bone.

a. **Bone resorption**- Calcitriol helps in bone resorption through PTH. Calcitriol receptors are present in osteoblasts . The formation of receptor - calcitriol complex on osteoblasts initiates cytokine signal that induces recruitment, differentiation and fusion of precursors into osteoclasts[30] .

b. **Bone mineralization**-. It also causes bone formation by the increasing proliferation of osteoblasts, secretion of alkaline phosphatase and osteoclastin synthesis[30].

ACTION ON KIDNEYS

Calcitriol increases the calcium and phosphate reabsorption in kidneys via enhancing calcium pumps in the epithelial cells of proximal and distal convoluted tubules. Calcitriol acts synergistically with PTH to increase calcium reabsorption from the kidneys. Vitamin D increases phosphate reabsorption from the kidneys[31].

ACTION ON PARATHYROID GLAND

Calcitriol has shown to regulate gene transcription and cell proliferation in the parathyroid glands[24]. Calcitriol has shown to decrease transcription of the PTH gene in vivo and in vitro[24].

NON-CALCEMIC OR NON-CLASSIC ACTIONS OF VITAMIN D

VDR are expressed in all the nucleated cells with 1-alpha hydroxylase activity in at least 10 different tissues apart from kidney[5]. Based on controlled observational studies in cells, tissues, transgenic mice and humans, it seems that functioning of all major tissues of the organism is modulated by vitamin D[24]. Vitamin D influences the functions of skin, skeletal muscles, the cardiovascular system, the immune system, placenta and deciduas and also the modulation of insulin resistance[32].

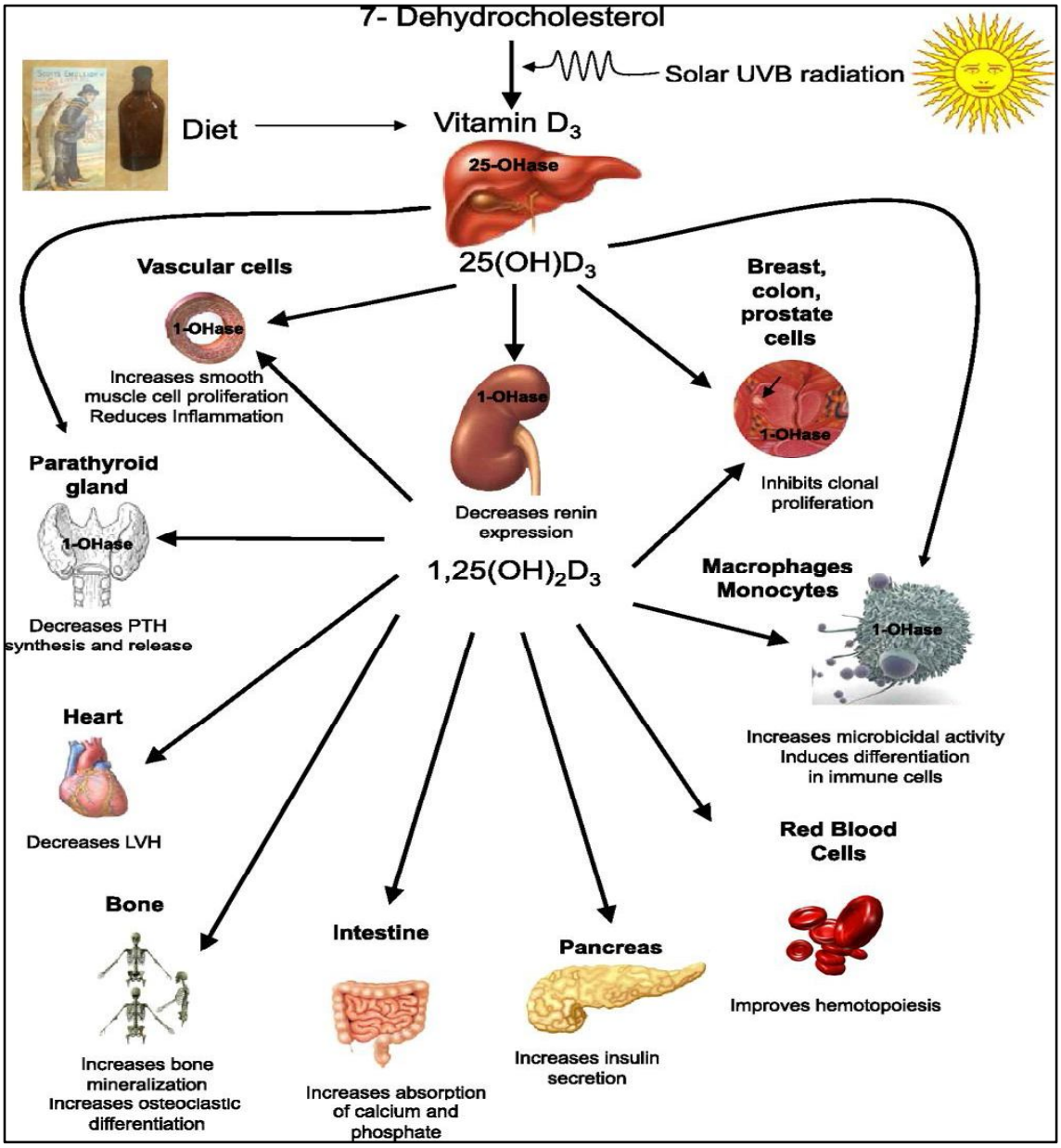
ACTION ON GLUCOSE METABOLISM

Endocrine beta cells , muscle, fat cells, which are important for glucose and energy metabolism are also targets for vitamin D. Vitamin D influences insulin secretion and insulin sensitivity, thereby maintains glucose homeostasis. The beta cells of pancreas express VDR and also show 1- alpha hydroxylase activity. It is found that diabetes is caused by the polymorphism affecting VDR[6].

The probable mechanisms by which vitamin D regulates the glucose homeostasis are:

a. Vitamin D enhances the insulin secretion by regulating intracellular calcium concentration [5].
b. Facilitates the action of calcium dependant endopeptidases which converts pro-insulin into active insulin, thereby increasing the release of insulin[5].
c. Enhances the action of insulin through regulation of the calcium pool in peripheral insulin target tissues.
d. Down regulates the production of pro-inflammatory cytokines like interleukin-2, TNF-alpha as well as interleukin-12. It induces regulatory T-lymphocytes and synthesis of anti - inflammatory mediators thereby prevents pancreatic β - cell destruction[5].

ACTIONS OF VITAMIN D



ACTION ON IMMUNE SYSTEM

All immune cells express a functional VDR and also can synthesise calcitriol using 1-alpha hydroxylase but are regulated by immune stimuli instead of calciotropic hormones[6]. The calcitriol enhances the natural defense against bacterial infections[6]. The calcitriol causes down regulation of the acquired immune system. Evidences report that VDD causes autoimmune diseases like type I diabetes [6].

ACTION ON THE SKELETAL MUSCLE AND SKIN

The VDR is expressed in myoblasts and present in lower concentration in mature striated muscle cells. Patients with vitamin D deficiency may develop severe myopathy which can be restored by appropriate vitamin D supplementation[24]. The combined presence of VDR, 1- alpha hydroxylase, 25-hydroxylase activity in the epidermis suggests that vitamin D contributes to keratinocyte growth and differentiation[24].

ACTION ON CELL PROLIFERATION AND CANCER

Exposure of calcitriol inhibits cell growth by interfering with signaling pathways initiated by prostaglandins, TGF and epidermal growth factor[6]. It has been found to regulate apoptosis and angiogenesis[6].

ACTION ON CARDIOVASCULAR SYSTEM

In vitro and in vivo exposure to calcitriol decreases the production of renin. It has been observed that a significant negative link between concentrations of 25(OH)D and blood pressure or plasma renin levels[6]. It is proposed that VDD is related to the development of metabolic syndrome[6].

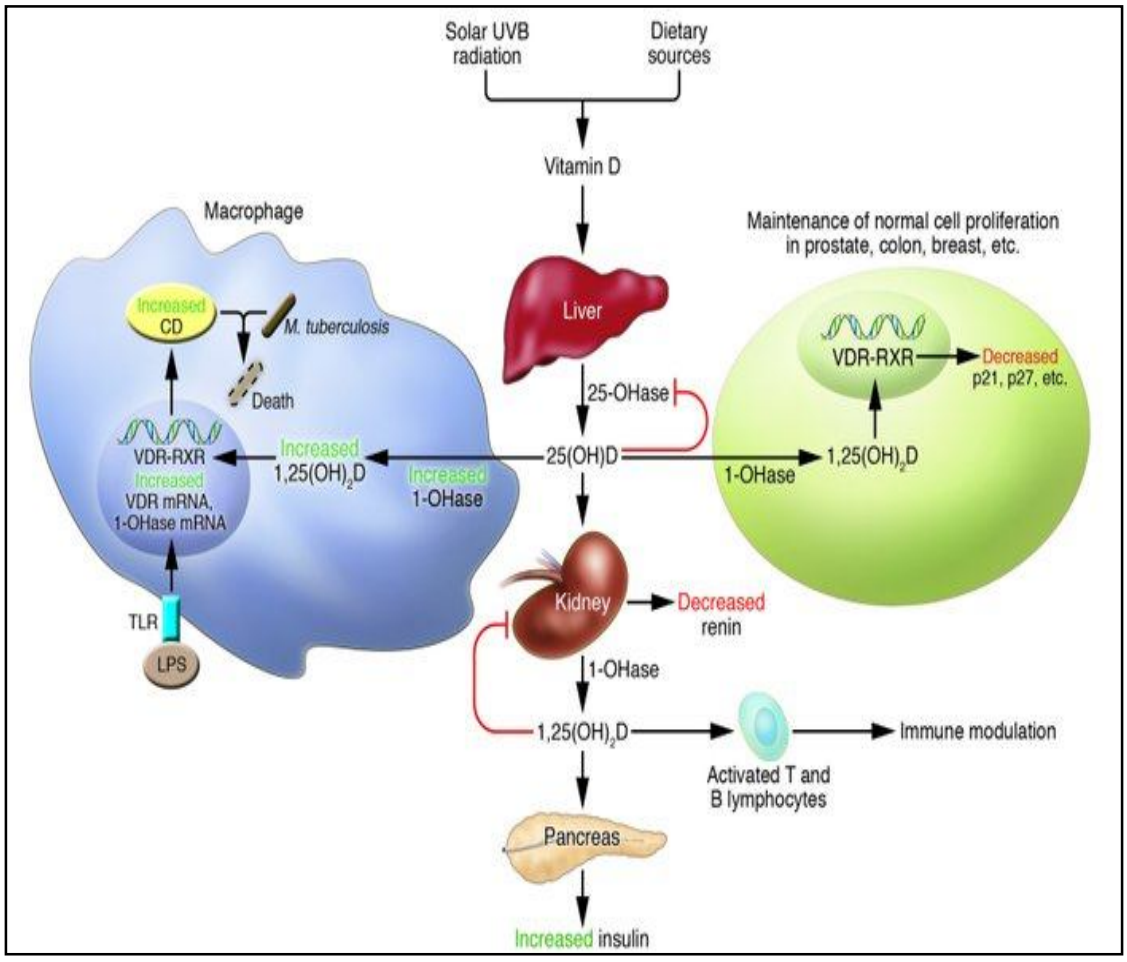
DEFICIENCY OF VITAMIN D AND DIABETES

Low blood levels of vitamin D have been reported to be linked to diseases like diabetes, osteoporosis, cancer, cardiovascular, neurodegenerative, infectious diseases, autoimmune diseases, fractures and poor physical function[5]. An association between deficiency of vitamin D and impaired insulin sensitivity in response to glucose has been demonstrated[32]. VDD causes impairment of insulin release leading to glucose intolerance. VDD predisposes to type1 DM, type 2 DM and GDM[5].

FACTORS RESPONSIBLE FOR VITAMIN D DEFICIENCY

Limited exposure to sunlight leading to inadequate synthesis of vitamin D, aging, inadequate dietary intake of vitamin D, obesity and darker skin[5].

EFFECT OF VITAMIN D ON IMMUNE SYSTEM



VITAMIN D DEFICIENCY AND TYPE 1 DM

The characteristic of type 1 DM is the damage to the pancreatic β-cell by an autoimmune reaction by cytokines and inflammatory agents. There is an inequity between pro- inflammatory and anti-inflammatory cytokines in type 1 DM[5].

The vitamin D receptors were identified in cells of immunological system like macrophages, lymphocytes which tempted the discovery of calcitriol as an immune modulator. The immune cells also have 1-alpha hydroxylase enzyme which helps in the conversion of 25(OH)D into active metabolite calcitriol[5]. Calcitriol induces the phagocytic capacity of macrophages and inhibits its antigen-presentation capacity. It also inhibits the production of pro-inflammatory cytokines[5]. It is recently evidenced that vitamin D suppresses IL-2 secretion in invitro studies[5].

In vivo administration of vitamin D causes suppression of IL-12 secretion and shift from CD4 T-helper type1 cells to CD4 T-helper type 2 cells. It has been found that vitamin D suppresses granulocyte macrophage–colony-stimulating factor and also down regulates IFN-gamma[5]. All these observations suggest that vitamin D is secreted by the immune cells and at the same time inhibits antigen presenting cells, proliferation of T cell, and secretion of cytokine. This vitamin also stimulates the synthesis of anti-inflammatory cytokine interleukin -4.

All these effects of the immune system are mediated through vitamin D receptors[5]. Thus vitamin D acts as an immune modulator and its deficiency leads to type 1 diabetes mellitus[5].

Vitamin D prevents the inhibitory effects of IL-1 beta or IFN –gamma on release of insulin. Roger Bouillon demonstrated that vitamin D treated pancreatic β-cells show reduced cytokines action[5].

DEFICIENCY OF VITAMIN D AND TYPE 2 DM

The characteristics of type 2 DM are altered insulin secretion with insulin resistance. Recent reports have demonstrated an association between VDD and type 2 diabetes[5]. In an observational cohort study, women taking higher vitamin D with calcium had 33% reduced risk of developing type 2 DM when compared to those taking reduced vitamin D. The pancreatic beta cells not only have VDR but also calbindin-D28K which is a calcium binding protein dependant on vitamin D[5]. This protein protects pancreatic β-cells against cytokine induced destruction, thereby prevents type 2 DM[5].

DEFICIENCY OF VITAMIN D AND ALTERED INSULIN RELEASE

Evidences suggest that pancreatic β-cells express VDR and 1-α hydroxylase activity which infer that vitamin D may contribute to insulin secretion[5].

Vitamin D has been reported to act by the possible mechanisms[5]:

- Acts directly on beta-cells to induce secretion of insulin by regulating the intracellular concentrations of calcium through voltage dependent calcium channels.
- Facilitates the action of endopeptidases in β-cells, that are dependant on calcium , thereby helps to convert pro-insulin into insulin.
- Enhances the action of insulin directly by facilitating insulin receptor expression in target tissues .
- Interferes with the generation of cytokines by enhancing the expression of the cytosolic protein calbindin in pancreatic beta cells. Calbindin protects the beta cells against the cytokine stimulated destruction[5].

VITAMIN D DEFICIENCY AND INSULIN RESISTANCE

Insulin resistance is the forerunner for type2 DM. Vitamin D mediates the transcriptional activation of insulin gene present on vitamin D responsive element and enhances insulin action directly . It also facilitates the expression of insulin receptors and the responsiveness of insulin for transport of glucose[5]. Vitamin D enhances secretion of insulin indirectly by regulating extracellular calcium, thereby ensuring an adequate intracellular cytosolic calcium via normal calcium influx through the cell membrane[5].

Ken C Chiu et al., reported the existence of direct correlation between 25-hydroxyvitaminD levels and insulin sensitivity[33]. Also, vitamin D promotes beta-cell survival by affecting the synthesis of cytokines[5]. The physiological activity of glucose transporters has been found to be impaired in diabetes. Insulin recruits glucose transporters from the cytoplasm to cell membrane. As insulin secretion is affected by vitamin D, its deficiency may lead to abnormal expression of glucose transporters[5].

VITAMIN D AND PREGNANCY

Vitamin D plays a crucial role in maternal and fetal health. It is the key modulator of metabolism of calcium[7]. In India, VDD is more common among pregnant and lactating women[8-10]. The prevalence of VDD in pregnancy has been reported to be about 70-90%[8,26] . During pregnancy, these changes provide the calcium needed for mineral accretion in fetal bone. About 25 to 30 gm of calcium reaches the fetal skeleton through placenta during last trimester of pregnancy[9].

The serum concentration of calcitriol increases up to two fold in pregnancy This increase starts at 10 to 12 weeks of pregnancy , reaches a upper limit in the last trimester of pregnancy and then returns to normal during lactation[32]. There is no change in 25(OH)D levels unless the intake or synthesis changes. The increased calcitriol synthesis may be due to increased 1-alpha hydroxylase activity in the placenta and decidua[7].

This increase in 1-alpha hydroxylase activity may be due to parathyroid hormone-related peptide (PTHrP)[7].

The serum calcium levels are maintained normal due to transfer of calcium to the fetus and some is lost in urine[32]. There is an increase in vitamin D binding protein during pregnancy[6]. Vitamin D affects dendritic cells of deciduas and macrophages, which stimulate T-regulatory cells in the maternal-fetal interface. Vitamin D inhibits the release of CD4 T-helper type1 cytokines and increases the release of CD4 T-helper type2 cytokines which dominate at implantation[11]. Vitamin D also helps in formation of decidual cells aiding in embryo implantation. It also facilitates the expression of a gene HOXA10 that contributes to the implantation of the embryo[11]. Totally, the data suggest that vitamin D supports implantation , fetal skeletal growth and inhibits synthesis of inflammatory cytokines[11].

Vitamin D deficiency is a pandemic occurring in both developed and developing countries. But the VDD in pregnancy has a special importance as it has a chance to affect the fetus. The following are the risk factors for VDD during pregnancy : lacking adequate exposure to sunlight, malabsorption of fat, vegetarian diet, drug therapy like steroids, anti-epileptic drugs, obesity and deeply pigmented skin[9].

Vitamin D deficiency is important during pregnancy as it is linked to adverse effects on mother like preeclampsia, obesity, insulin resistance, and GDM. Adverse outcomes of vitamin D deficiency in newborns are LBW, neonatal rickets, diabetes , bronchial asthma and hypocalcaemia in neonates[9].

It is observed that decreased levels of 25-hydroxyvitaminD contributes to glucose intolerance while higher serum 25-hydroxyvitaminD levels enhances insulin sensitivity . VDD in pregnancy significantly raises the possibility of GDM[11].

CLASSIFICATION OF VITAMIN D DEFICIENCY

The Endocrine society's clinical guidelines for defining vitamin D status[34]

- Deficiency - Serum 25-hydroxyvitaminD concentrations less than 20ng/ml
- Insuffiency - 25-hydroxyvitaminD levels between 21-29 ng/ml
- Sufficiency - 25-hydroxyvitaminD levels between 30 to 100 ng/ml.

Lips criteria for Vitamin D deficiency[35]

- Mild deficiency - between 10.1 -20 ng/ml
- Moderate deficiency - between 5.1 - 10 ng/ml
- Severe deficiency - less than 5 ng/ml.

VITAMIN D REQUIREMENT IN PREGNANCY

The vitamin D adequacy in pregnancy is not clearly known. The National osteoporosis Foundation had recommended 400- 800 IU of vitaminD during pregnancy[9]. Daily intake of 1500–2000 IU of vitamin D may help in maintaining a serum concentration above 30ng/ml during pregnancy. Requirement may also may raise upto 6000 IU/day[36,37].

MEASUREMENT OF VITAMIN D

Nowadays specific assay techniques for estimation of vitamin D metabolites are developed which has revolutionized the laboratories[38]. There is an apprehension in selecting the metabolite to be measured, which depends upon the their half-life . The half-life (t ½) of total vitamin D is about 24 hours which depends upon the exposure to sunlight and ingestion of vitamin D. The half-life for calcitriol is about 4 hours and its synthesis is strictly regulated by body's calcium need. The half-life of 25-hydroxyvitaminD is longer about 2-3 weeks. This long t½ of 25-hydroxyvitaminD is marker of vitamin D stores. Also 25-hydroxyvitaminD synthesis is not regulated significantly and depends on substrate concentration. Hence, estimation of 25(OH)D gives an important clinical assessment of body's vitamin D status[38]. True deficiency of vitamin D would be evident only by measuring levels of 25(OH)D[9].

METHODS AVAILABLE FOR MEASURING 25-HYDROXYVITAMIN D

In 1971, two competitive protein binding assays were introduced for measurement of serum 25(OH)D. The solvent system used for extraction and also incubation times differed in both assays[38]. In 1977, the measurement of 25(OH)D was done using direct ultraviolet detection assay. This method helped in separate estimation of 25-hydroxyvitaminD_2 and 25-hydroxyvitaminD_3 but it needed expertise with equipment which is available in research laboratories only[38].

In 1985, to simplify the procedure of extraction of 25(OH)D , radio immunoassay was developed. Radioimmunoassay (RIA) recognized other metabolites of vitamin D like 25(OH)D_2, 25(OH)D_3, 24, 25 - dihydroxy vitamin D, and 25,26- dihydroxy vitamin D[38]. There are two enzyme- linked immune sorbent assays (ELISA) also for measurement of 25(OH)D. DiaSorin has introduced a chemiluminescent assay for 25(OH)D which is fully automated[38] . This assay does not need pretreatment of samples. The time taken for the assay is 40 minutes and about ninety samples can be analysed per hour. The last method is the Liquid chromatography - tandem mass spectrometry (LC-MS/MS) has proved to be the gold standard[34]. This tecnique can separate and quantitate the two metabolites of vitamin D[38]. But LC-MS/MS encounters issues with ion suppression[38].

VITAMIN D AND GDM

GDM is intolerance of carbohydrate metabolism of variable severity with first recognition or onset during pregnancy[1]. Gestational Diabetes is a form of type 2 diabetes mellitus discovered during pregnancy. VDD during pregnancy is found to be linked with GDM[39]. The inflammation of the placenta and the insulin receptors contributes to the development of GDM which is proposed to be regulated by vitamin D[20].

The classic mechanism by which vitamin D regulates glucose homeostasis is that it enhances calcium influx of β cells of the pancreas leading to depolarization mediated secretion of insulin[40]. Vitamin D facilitates the action of endopeptidases in beta-cells , which converts pro-insulin to insulin[5]. But, other Vitamin D dependent mechanisms studied recently are :

- Increased expression of genes like peroxisome proliferator activated receptor - gamma (PPAR γ) , the coactivator (PGC1α) concerned with glucose metabolism[41].
- Upregulation of the insulin receptor substrate -1 (IRS-1) in the muscles [42].
- Facilitating GLUT-4 action in adipocytes and its recruitment to cell membrane [43].

- Maintaining the normal function of enzymes like hexokinase, glucose- 6-phosphatase[44].
- Vitamin D acts as a potent immuno suppressor that down-regulates the transcription of pro inflammatory cytokine genes and induces synthesis of anti-inflammatory cytokines thereby prevents β-cell damage[5].

When there is vitamin D deficiency during pregnancy , the synthesis and secretion of insulin, protection of beta cells against inflammatory cytokines and insulin sensitivity may be affected thereby would alter the glucose homeostasis which may lead to the development of GDM[5].

STUDIES RELATED TO VITAMIN D DEFICIENCY AND GDM

Many studies were performed to reveal the association between VDD and GDM. Sayid shafi zuhur et al., estimated vitamin D concentrations in 234 GDM women along with 168 normal pregnant women to find the link between low 25-hydroxyvitaminD levels and GDM in Turkish pregnant women . The results showed a high likelihood of GDM in women with severely deficient maternal serum 25(OH)D[45].

Cho GJ et al., performed a study on 40 women with normal pregnancy and 20 GDM women and inferred that 85% GDM women had VDD with serum concentrations of 25-hydroxyvitaminD below 20ng/ml. Women with GDM showed decreased vitamin D levels in contrast to normal pregnant women ($p < 0.01$)[46].

Cuilin Zhang et al., analysed vitamin D levels in 57 GDM women and 114 normal pregnant women. The findings of the study showed that the maternal serum 25(OH)D concentrations were significantly decreased in women with GDM than the women with normal pregnancy ($p<0.001$) as well as there was 1.29 times increased risk of gestational diabetes for every 5ng/ml fall in 25-hydroxyvitaminD level[47].

Ahmed E1 Lithy et al., observed that women with lower vitamin D concentrations were prone for poor glycemic control and higher levels of HbA1c[48]. The systematic review and meta-analysis on 2146 participants by Y.H.M.Poel et al., reported, a statistically inverse correlation between 25-hydroxyvitaminD levels and GDM incidence[49].

The concentrations of 25-hydroxyvitaminD estimated during second trimester by Heather H Burris et al., negatively correlated with the blood glucose levels[50]. The lower levels of serum 25(OH)D may be linked with increased risk of GDM[50] and similar findings were reported by Joong Sik Shin et al [11].

Soheilykhah et al., in a study observed that serum 25-hydroxyvitaminD levels were lower in GDM (24nmol/L) as compared to normal pregnancy (32 nmol/L) . They also reported that women with VDD had 2.66 fold increased possibility of GDM when compared to control group[16].

Maghbooli et al., in their study illustrated that the concentrations of 25-hydroxyvitaminD positively correlated with insulin sensitivity which concluded that VDD may contribute to insulin insensitivity in pregnancy and also severe VDD was more prevalent in GDM than normoglycemic subjects [17].

Napartivaumnuay N et al., in their study on 197 Thai pregnant women reported that GDM cases had decreased vitaminD levels compared to normal pregnancy[51]. Olmos-Ortiz et al., had stated that glucose intolerance and possibility of GDM was high in women with decreased 25-hydroxy vitaminD concentrations[52].

Lacroix M et al., in his cohort study observed that decreased 25-hydroxyvitaminD concentration at first trimester is an independent cause for GDM and linked to higher insulin resistance at second trimester of pregnancy[53].

Arnold DL et al., concluded that early pregnancy concentrations of serum 25-hydroxyvitaminD_3 are inversely correlated with the development of GDM and also observed a 14% reduction of GDM risk for a 5ng/ml raise in vitaminD concentration[54].

Madhu Jain et al., observed that there was an higher frequency of VDD in early pregnancy and the women with GDM showed lower concentration of 25-hydroxyvitaminD (11.93 $\pm$ 3.42 ng/ml) compared to normal pregnancy (22.26 ±15.28 ng/ml) with p <0.0001 and found significant[21].

Kaushal M and Magon N had reported that vitamin D influences insulin secretion, and also low concentrations of vitamin D contributes to insulin resistance that leads to glucose intolerance and then GDM[9].

Wang O et al., studied the association between VDD and development of GDM in 200 GDM cases and 200 women with normal pregnancy in China. They stated that low maternal serum vitamin D concentrations were linked to insulin resistance and possibility of GDM[55].

The study by Aanchal Sablok et al., to find the effect of vitamin D substitution in pregnant women as well as to evaluate its association with feto-maternal outcomes illustrated that likelihood of GDM is greater in pregnant women with lower vitamin D concentrations and supplementation of vitamin D had reduced such risks[56].

The study of Senti J, Thiele DK and Anderson CM illustrated that VDD in pregnancy was linked with the markers of altered glucose homeostasis[57]. CV Harinarayan and Shashank R Joshi in their article, had explained that VDD leads to development of hypertension, stroke, **diabetes**, depression, chronic pain, autoimmune diseases, myopathies, and birth defects[58].

It is evident from the study of Mozaffari- Khosravi H et al., that single injection of vitamin D improved the maternal vitamin D status along with insulin sensitivity in women with GDM[59].

Rudnicki and Molsted-Pederson demonstrated a transient decrease in fasting blood glucose levels after intravenous injection of calcitriol in GDM cases, which proved that vitamin D may directly increase glucose absorption in the cells by increasing insulin sensitivity[15] and thereby proved the influence of vitamin D in glucose metabolism.

All these studies make apparent that vitamin D is involved in insulin secretion and function, thereby playing a major role in glucose metabolism.

There are studies which show no relationship between VDD and development of GDM. Mahlatse Makgoba et al., carried out a study in 90 GDM cases and 158 normal pregnant women . The study reported no evidence for link between first trimester vitaminD levels and consequent risk of GDM[60].

Park S et al., in a study on 523 pregnant women to assess the risk of development of GDM in pregnant women with VDD. The study proved no relationship between 25(OH)D values and possibility of GDM [61]. The same findings were observed by Rodriguez and co-workers[62].

Hulya Parildar et al., performed a study on 44 GDM cases as well as 78 healthy women with normal pregnancy to find the frequency of VDD in women with GDM . They observed no significant link between vitamin D concentration and fasting blood glucose levels, HbA1c levels and levels of insulin[63].

Anna Pleskacova et al., aimed to find the relationship between mid gestational and postpartum vitaminD status and glucose tolerance. The results showed no decrease in 25-hydroxyvitaminD levels in women with GDM[64]. Polina popova et al., illustrated that decreased concentrations of 25- hydroxyvitaminD were not statistically related to GDM [65]. The same findings were reported by Schneuer FJ et al[66].

Hence the current study was designed to assess the maternal vitaminD status in pregnancy as well as to determine if there is any link between the 25-hydroxyvitaminD levels and GDM. As VDD is a modifiable cause of GDM, it is worth determining maternal vitamin D status in pregnancy and its supplementation would prevent maternal complications like obesity, GDM, preeclampsia and other related fetal adverse outcomes.

MATERIALS & METHODS

MATERIALS AND METHODOLOGY

STUDY DESIGN

This is a Cross Sectional study.

STUDY PLACE

The study was performed in the department of Physiology along with the department of Obstetrics and Gynaecology, Coimbatore Medical College and Hospital, Coimbatore.

STUDY PERIOD

The study period extended from July 2014 to June 2015.

STUDY SUBJECTS

A total of 100 pregnant women between 20 - 35 years of age and Body Mass Index (BMI) of 18.5 – 29.9 Kg/m^2 were selected for the study. Fifty women diagnosed having GDM by 75gm OGTT with blood sugar levels of $\geq$ 140mg/dl after 2 hours of oral glucose were considered as GDM group. The control group comprised of fifty women with normal glucose levels after 75 gm OGTT .

INCLUSION CRITERIA:

- Fifty women diagnosed having GDM who were age as well as BMI matched – GDM group
- Fifty normal pregnant women who were also age as well as BMI matched – control group
- Both primigravida and multigravida were included.
- Pregnant women having antenatal records with height and weight recorded at first booking visit (around 6-8 weeks of pregnancy) were included.
- Pregnant women were selected for estimation of serum 25-hydroxy vitaminD concentrations during 24 to 28 weeks of gestation.

EXCLUSION CRITERIA:

- Pre-gestational diabetes
- Hypertension
- Twin pregnancy
- Obese women (BMI >30Kg/m^2)
- Women with other systemic diseases like hypothyroidism, autoimmune diseases, liver and renal diseases.
- H/o drug intake like steroids and anti-epileptic drugs

HISTORY TAKING

MATERIALS USED FOR THE STUDY:

- Proforma - To obtain a detailed history and clinical examination findings.
- Fetoscope - to determine the fetal heart sound.
- Auto analyser – for analysis of blood sugar level.
- Immuno analyser - to measure total 25(OH)D levels.

METHODOLOGY :

After obtaining clearance from the institutional ethical committee, the cases and controls were selected. The cases and controls were explained about the procedure in detail and informed written consent was obtained.

The study protocol consists of :

HISTORY TAKING AND CLINICAL EXAMINATION:

Detailed history was elicited from the cases and controls to rule out signs and symptoms of pre-gestational diabetes, cardiovascular diseases, liver and kidney diseases and any intake of drugs. The last menstrual period (LMP) was noted and weeks of pregnancy was calculated using Naegele's rule (add 7 days to the first day of LMP and count back three months). General examination was done.

OBSTETRIC EXAMINATION ASSESSING WEEKS OF PREGNANCY

RECORDING OF FETAL HEART SOUND

Obstetric examination was done to confirm the weeks of pregnancy. Fetal heart sound was determined by using fetoscope. A thorough clinical examination of all systems were done.

BODY MASS INDEX (BMI) - MEASUREMENT

The height as well as weight recorded during the first visit of pregnancy (6-8 weeks of gestation) were obtained from the antenatal records and BMI was calculated.

BODY MASS INDEX CALCULATION:

BMI was calculated by using Quetelet's index.

BMI = Weight in Kilogram / Height in square meters.

The subjects were selected with a BMI between 18.5 to 29.99 kg/m^2 which included women with normal BMI (18.5 to 24.99) and overweight (25 to 29.99) according to WHO criteria. The obese women (BMI >30) were not included in the study.

BLOOD INVESTIGATIONS

a. 75 gm OGTT – WHO CRITERIA[22]

This is a 75 gm, 2 hour OGTT[22]. This investigation was done to standardize the diagnosis of GDM. A cut-off value for diagnosing GDM is a plasma glucose level of $\geq$ 140 mg% after 2 hours oral glucose according to WHO criteria.

The test was done irrespective of the last meal. The test was done at 24 to 26 weeks of pregnancy. In the antenatal clinic, the pregnant women were given a 75 gram glucose load orally. Then a blood sample was collected from a peripheral vein after 2 hrs and the estimation of plasma glucose was done using Glucose Oxidase - Peroxidase (GOD - POD) technique.

Principle of GOD-POD method : Glucose is oxidized to gluconate and hydrogen peroxide by glucose oxidase . This hydrogen peroxide yields a red quinoeimine dye by oxidative coupling with phenol and 4 amino – anti pyrene in the presence of peroxidase. The absorbance takes place at 505nm and is directly proportional to the amount of glucose in the sample.

COLLECTION OF BLOOD SAMPLE FOR ESTIMATION OF TOTAL SERUM 25(OH)D

b. MEASUREMENT OF TOTAL SERUM 25(OH)D LEVELS

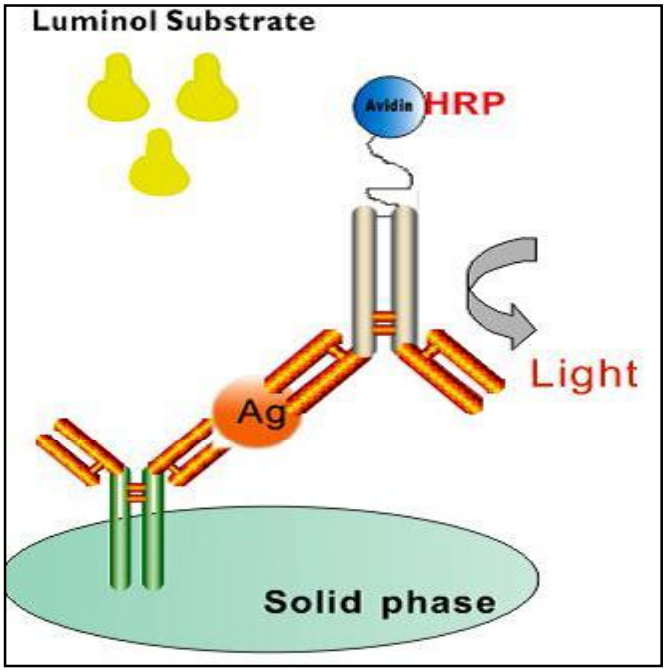
Antecubital vein in the front of forearm was selected for venous blood collection. The skin over the vein was cleaned with spirit and allowed to dry. Then a disposable sterile needle fitted with 5 ml syringe was introduced into the vein and 4ml of blood was collected and poured into separate containers having anticoagulants. The serum was separated by centrifuging the blood to 3000 rpm for 5 minutes. The serum was used to estimate total 25(OH)D level. This was done by fully automated Chemiluminescence assay. (ADVIA centaur immune assay technique).

Fully Automated Chemiluminescence Immuno Assay (CLIA)[67]:

The fully automated CLIA is a direct competitive method for analysis of total serum 25-hydroxyvitaminD

Principle: Chemiluminescence is emission of light formed during a chemical reaction. The chemiluminescent labels are isoluminol, acridinium esters and luminal. It involves oxidation of these chemiluminescent molecules. The oxidants responsible for the above reactions are hydrogen peroxide, hypochlorite, oxygen. Light is emitted from the excited product after oxidation. The enzymes which catalyse these reactions are horse radish peroxide, alkaline phosphatase. The photon counter reader is used for estimating the light signals the relative luminosity values (RLU)[67].

CHEMILUMINESCENCE IMMUNOASSAY



ADVIA CENTAUR CHEMILUMINESCENCE IMMUNO ASSAY

The ADVIA centaur CLIA was employed for estimation of total 25-hydroxyvitaminD. This technology has proved to be the reliable investigation for estimating the total 25-hydroxyvitamin D as it is highly sensitive and has nil radioactivity . The CLIA employs an anti-fluorescein antibody taken from mouse. It is a monoclonal antibody bound to para magnetic particles. The assay also utilises an anti-25 –hydroxyvitamin D antibody complexed with acridinium esters and an analog of vit D tagged with fluorescein. Photomultiplier is used to analyse the light signal as RLU which is inversely proportional to the levels of 25-hydroxyvitaminD[68].

STATISTICAL ANALYSIS

STATISTICAL TOOLS

The data obtained from the current study were documented in a Master Chart. Data analysis was done using Epidemiological Information Package (EPI 2010) in the computer, given by the Centre for Disease Control at Atlanta.

Employing the above software , calculations of the mean, the range, the frequencies, percentages, standard deviations(SD), chisquare and 'p' values were done. Student's 't' test was employed to analyse the significance of difference between the quantitative variables (age, height, weight, BMI, 25-hydroxyvitaminD and OGTT). Fisher's chi square test is used for qualitative variables (diet, residence, family history, gravida). A significant association is considered only when the 'p' value is less than 0.05.

Microsoft Power point was employed to prepare graphs.

RESULTS

RESULTS

A: PARAMETERS STUDIED BETWEEN THE GDM AND THE CONTROL GROUP

Table A1 : AGE DISTRIBUTION BETWEEN THE GDM AND THE CONTROL GROUP

Age	GDM Group		Control group	
	No	%	No	%
20 -25 yrs	19	38	30	60
26 – 30 yrs	24	48	14	28
30 -35 yrs	7	14	6	12
Total	50	100	50	100
Age (years)				
Range	20 – 35 yrs		20 – 35 yrs	
Mean	26.6 yrs		25.3 yrs	
SD	3.2 yrs		3.8 yrs	
'p'	0.0591 Not significant			

There is no significant difference in age distribution between GDM group and control group.

Figure 1 : AGE DISTRIBUTION

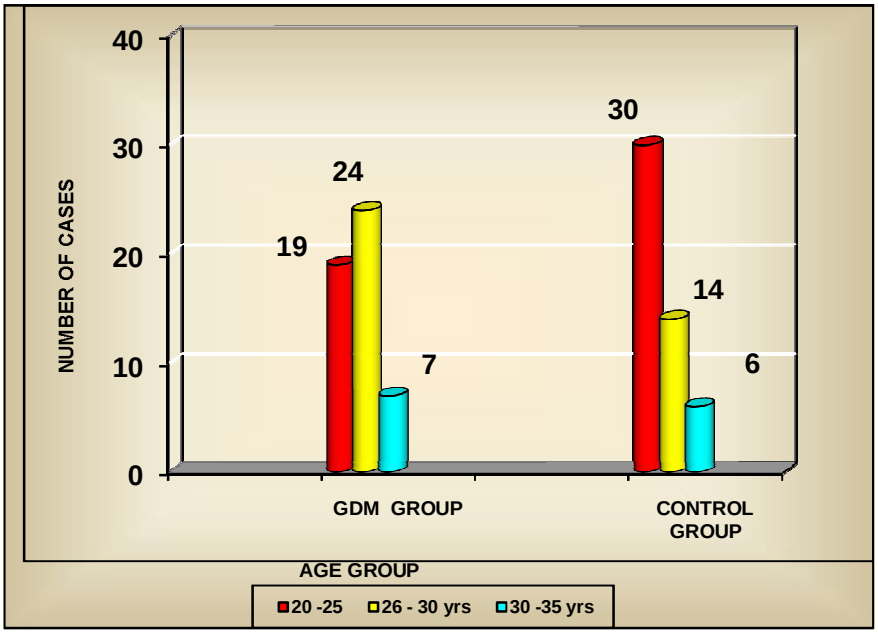


Figure 2 : MEAN AGE

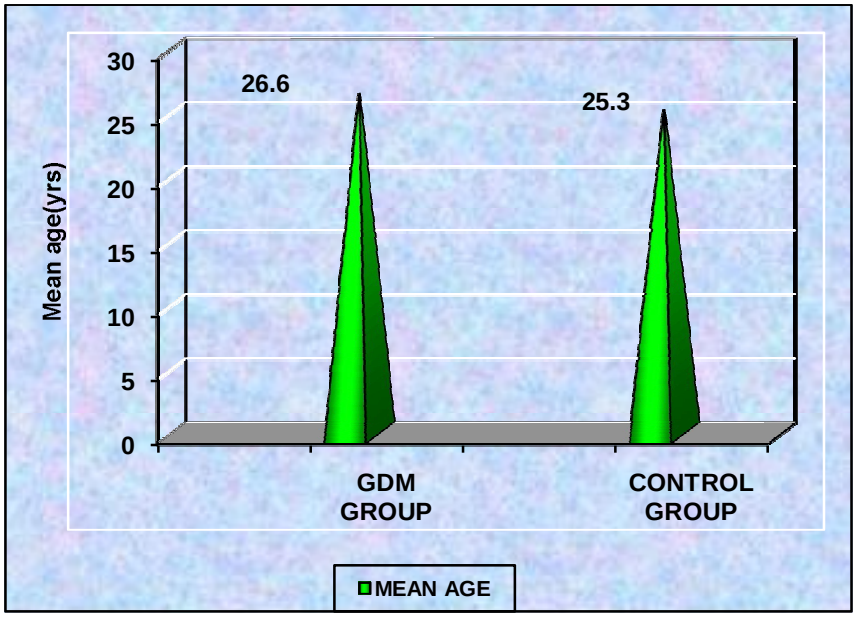


Table A2 : DISTRIBUTION OF RESIDENCE BETWEEN GDM GROUP AND CONTROL GROUP

Residence	GDM Group		Control group	
	No	%	No	%
Rural	12	24	11	22
Urban	38	76	39	78
Total	50	100	50	100
'p'	1.0 Not Significant			

There is no significant difference in urban and rural residential distribution between GDM group and control group.

Figure 3 : RESIDENCE

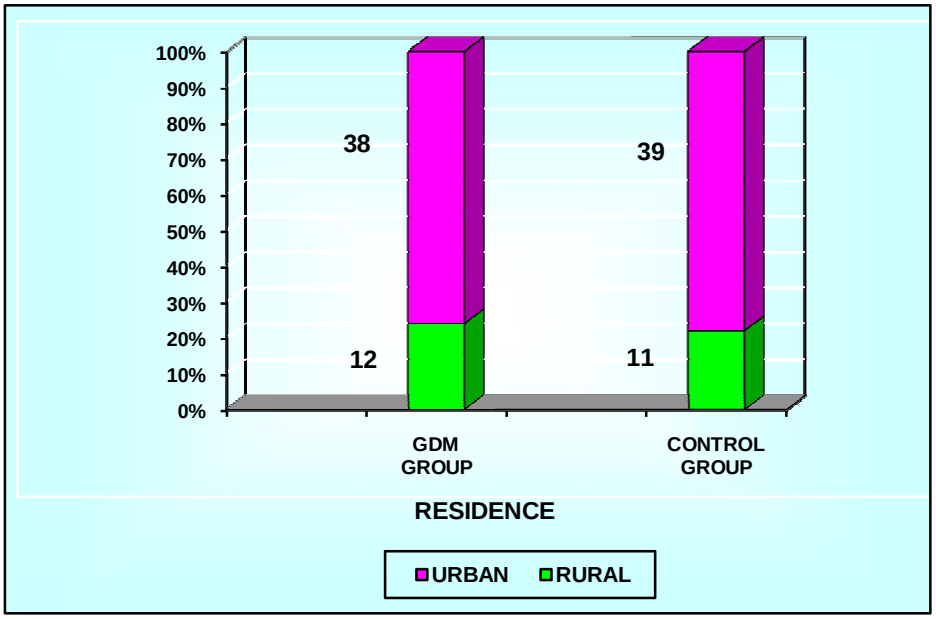


Table A3 : DIET HABITS BETWEEN GDM GROUP AND CONTROL GROUP

Diet Habit	GDM Group		Control group	
	No	%	No	%
Vegetarian	27	54	24	48
Non vegetarian	23	46	26	52
Total	50	100	50	100
'p'	0.6891 Not Significant			

The above results show no statistical difference in diet habits between the GDM and control groups.

Figure 4 : DIET HABITS

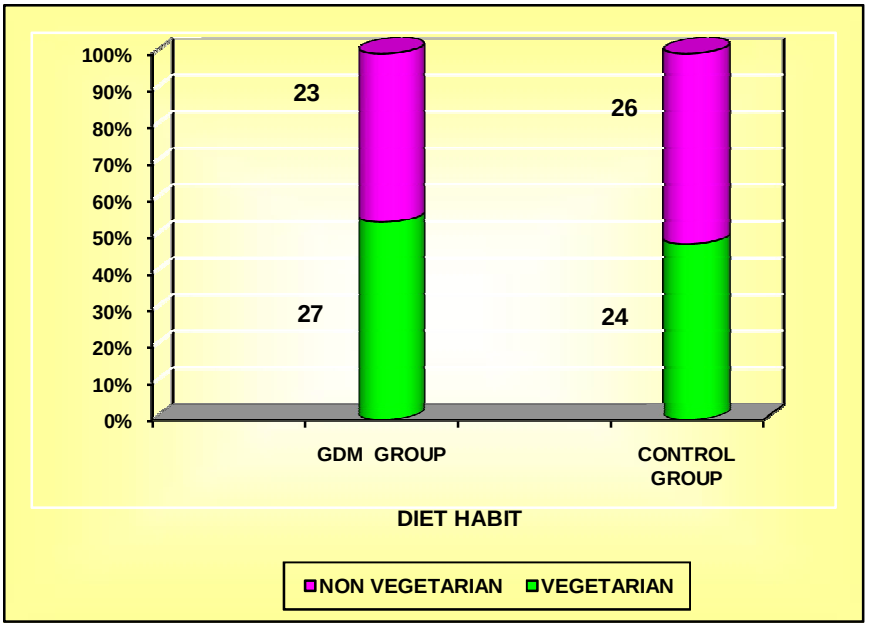


Table A4 : WEIGHT / HEIGHT / BMI DISTRIBUTION BETWEEN THE GDM GROUP AND CONTROL GROUP

Variables	GDM Group		Control Group		'p'value
	Mean	SD	Mean	SD	
Weight (kgs)	61.8	4.6	60.1	5.9	0.1188 Not Significant
Height (cms)	153.8	4.9	153.7	4.0	0.9288 Not significant
BMI(kg/m^2)	26.23	2.12	25.49	2.61	0.1243 Not Significant

Both the groups show no significant variation in the distribution of BMI

Figure : 5 BMI DISTRIBUTION

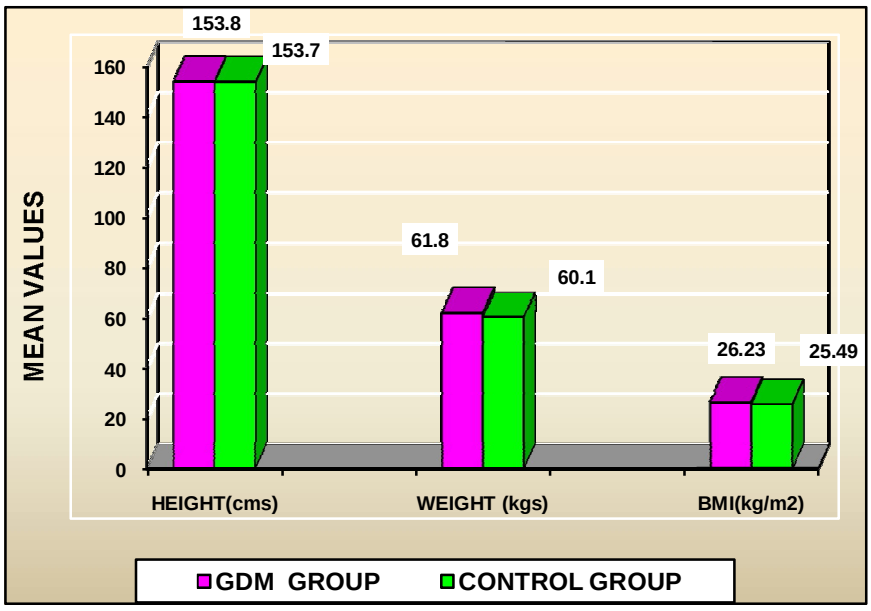


Table A5: FAMILY HISTORY OF DM

Family h/o of DM	GDM Group		Control group	
	No	%	No	%
Yes	13	26	11	22
No	37	74	39	78
Total	50	100	50	100
'p'	0.8149 Not Significant			

Both the groups show no statistical difference in family history of diabetes.

Figure : 6 FAMILY HISTORY OF DIABETES

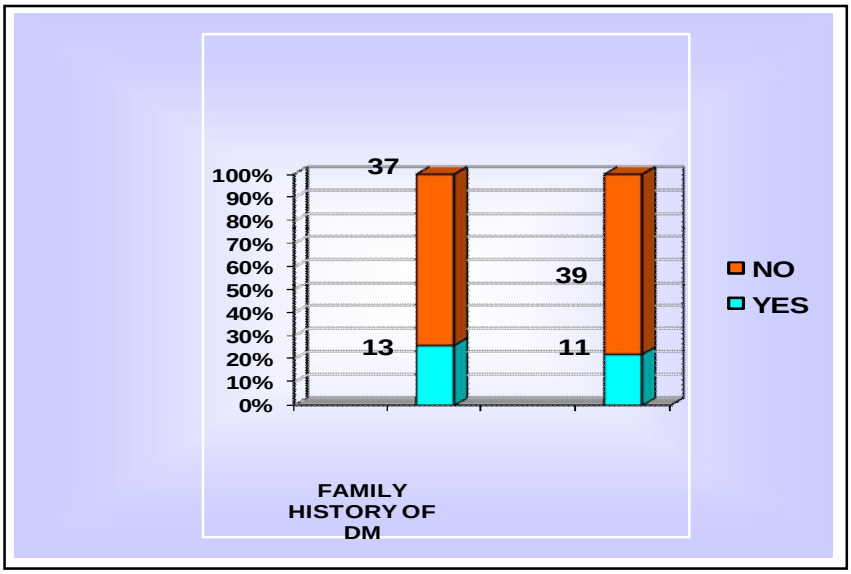


Table A6: DISTRIBUTION OF GRAVIDA BETWEEN GDM GROUP AND CONTROL GROUP

Gravida	GDM Group		Control group	
	No	%	No	%
Primi	26	52	27	54
Multi	24	48	23	46
Total	5050	100	50	100
'p'	1.0 Not Significant			

There is no significant difference in distribution of primigravida and multigravida between both the groups.

Figure :7 GRAVIDA DISTRIBUTION

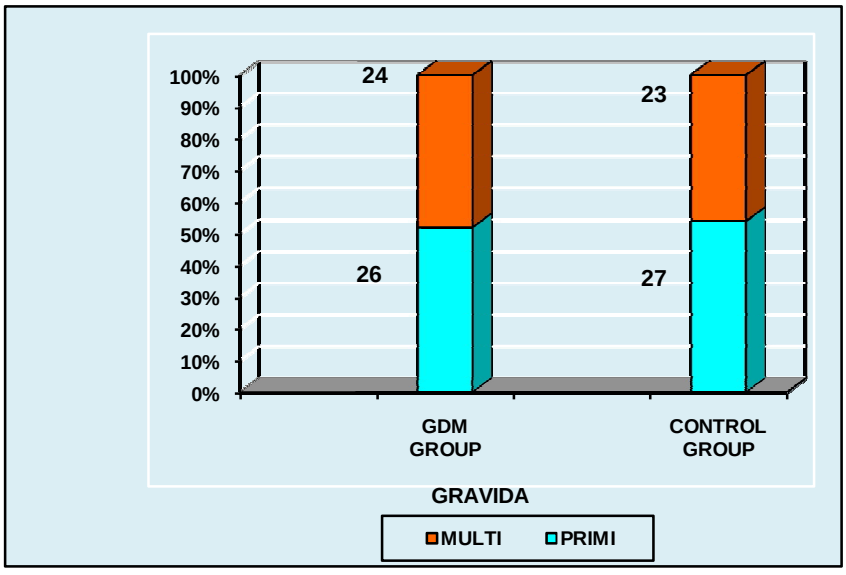


Table A7 : WEEKS OF PREGNANCY BETWEEN GDM GROUP AND CONTROL GROUP

Parameter	Weeks of Pregnancy	
	GDM Group	Control Group
Range	24 – 28 weeks	24 – 28 weeks
Mean	24.92 weeks	25.04 weeks
S.D.	1.03 weeks	1.12 weeks
'p'	0.5786 Not significant	

There was no statistical difference in weeks of pregnancy between both the groups .

Figure:8 WEEKS OF PREGNANCY

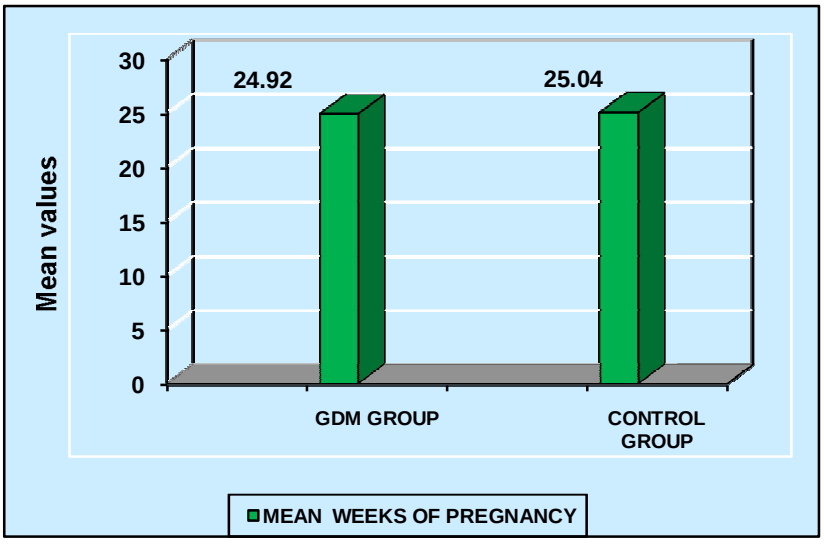


Table A8: OGTT VALUES BETWEEN GDM AND CONTROL GROUP

Parameter	OGTT (mg/dl)	
	GDM Group	Control Group
Range	144 – 273	84 – 136
Mean	183.1	113.1
S.D.	30.3	14.1
'p'	< 0.0001 Significant	

Oral Glucose Tolerance test shows a significant difference in both the groups. The mean blood sugar level in GDM group was 183 ± 30.3 mg/dl and in control group was 113.1 ± 14.1 mg/dl. The p value was found significant ($p < 0.0001$).

Figure:9 OGTT VALUES BETWEEN GDM AND CONTROL GROUP

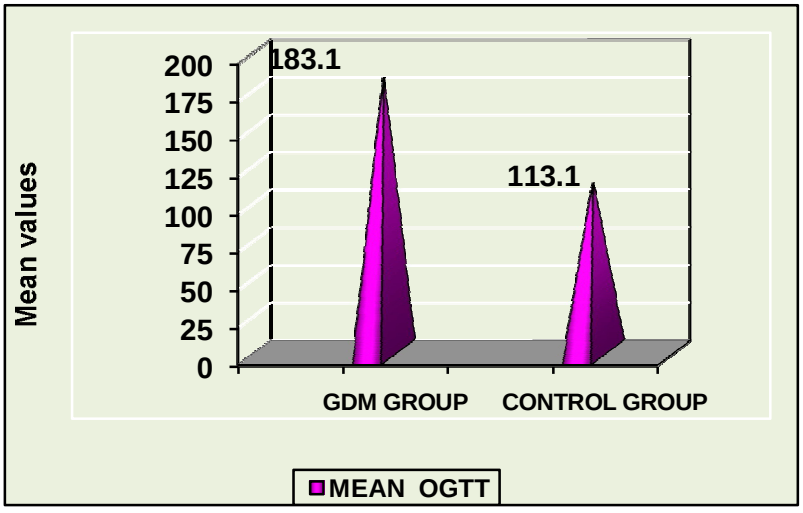


Table A9 : VITAMIN D LEVELS BETWEEN GDM GROUP AND CONTROL GROUP

Vit D status (ng/ml)	GDM Group		Control group	
	No	%	No	%
Severe deficiency (< 5)	7	14	-	-
Moderate deficiency (5.1 – 10)	23	46		
Mild deficiency (10.1 - 20)	12	24	2	4
Insufficiency (20.1 – 30)	5	10	12	24
Sufficient levels (> 30)	3	6	36	72
Total	50	100	50	100
Vitamin D (ng/ml)				
Range	4.56 – 32.26		17.42 – 44.42	
Mean	11.78		31.48	
SD	7.86		5.01	
'p'	< 0.0001 Significant			

Women in the GDM group showed significantly lower concentrations of vitamin D levels compared to women with normal pregnancy.

Figure:10 25-HYDROXYVITAMIN D CONCENTRATIONS IN THE GDM AND CONTROL GROUP

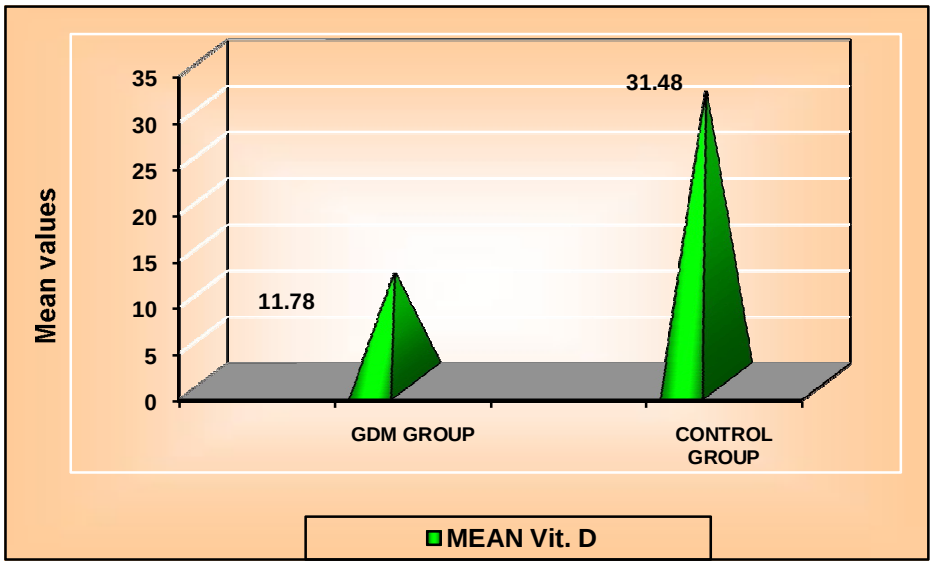
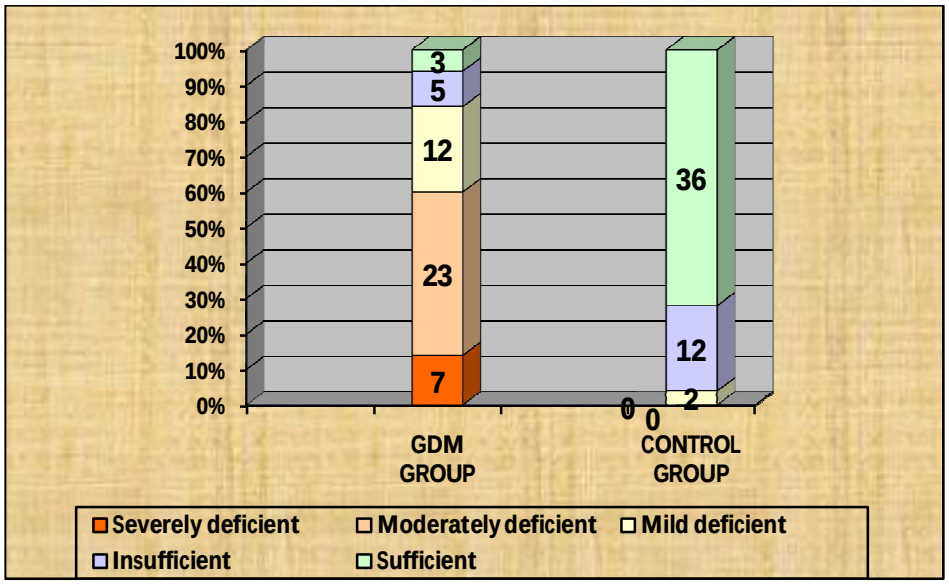


Figure :11 VITAMIN D STATUS IN GDM GROUP AND CONTROL GROUP



B : ASSOCIATION BETWEEN VITAMIN D AND OTHER VARIABLES IN GDM GROUP

Table B1 : AGE & VITAMIN D LEVELS

Age Group	Total 25(OH)D (ng/ml)	
	Mean	S.D.
20 to 25 yrs	15.98	9.21
26 – 30 yrs	9.97	6.17
31 -35 yrs	6.6	1.84
'p' value	0.0054 Significant	

In the GDM group, the above results show significant link between the age and vitamin D concentrations. As the age increases, the vitamin D levels decrease.

Figure :12 AGE & VITAMIN D LEVELS

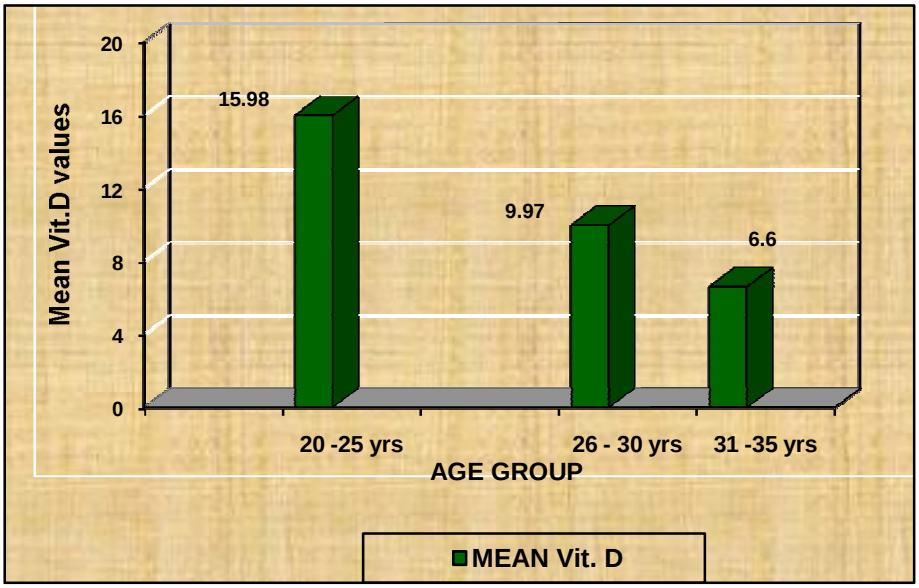


Table B2 : DISTRIBUTION OF RESIDENCE & VITAMIN D LEVELS

Residence	Vit D (ng/ml)	
	Mean	S.D.
Rural	11.01	5.36
Urban	12.03	8.54
'p'	0.7009 Not significant	

There is no statistical difference in levels of 25-hydroxyvitamin D between the rural and urban population in this study.

Figure 13 : RESIDENCE & VITAMIN D LEVELS

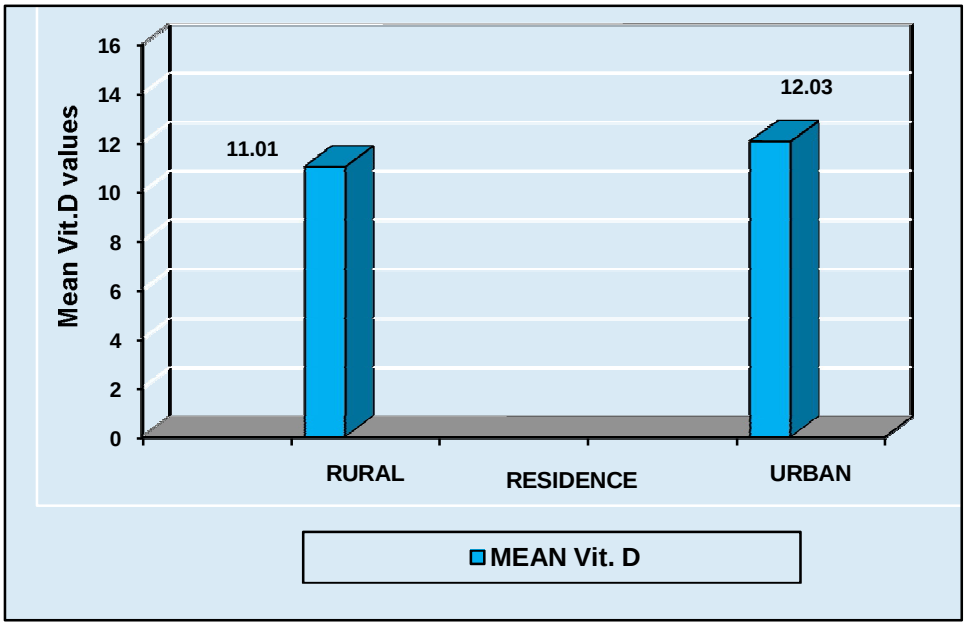


Table B3 : DIET HABIT & VITAMIN D LEVELS

Diet Habit	Vit D (ng/ml)	
	Mean	S.D.
Vegetarian	13.23	9.09
Non vegetarian	10.55	6.54
'p'	0.2338 Not Significant	

Above results show no significant variation in vitamin D status among vegeterians and non-vegeterians in the GDM group.

Figure: 14 DIET HABIT & VITAMIN D LEVELS

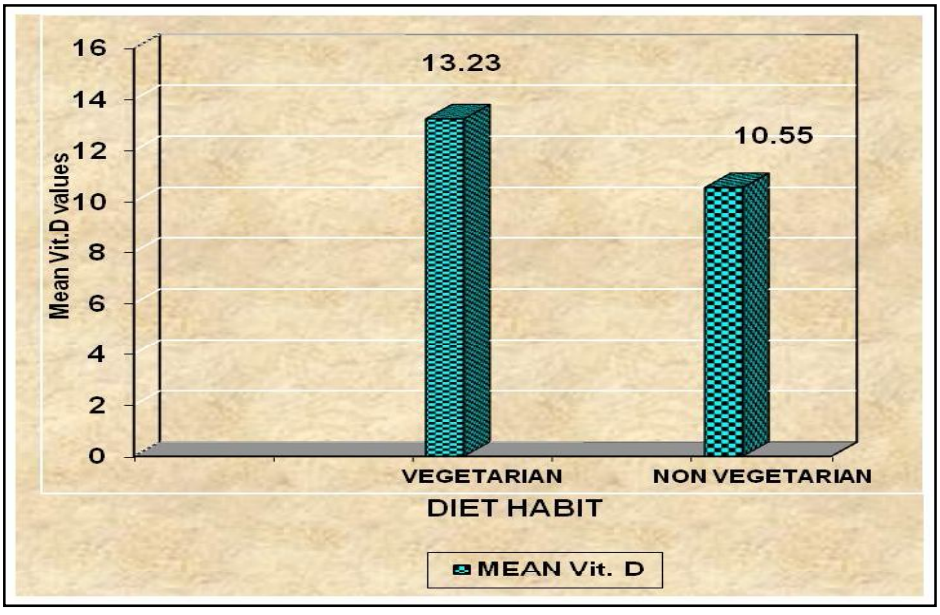


Table B4 : BMI & VITAMIN D LEVELS

BMI (Kg/m^2)	Vit D (ng/ml)	
	Mean	S.D.
18.5 to 24.99	14.78	5.22
25 – 29.9	10.83	8.35
'p'	0.1305 Not significant	

The above results show no statistical link between BMI and vitaminD in GDM group.

Figure :15 BMI AND VITAMIN D LEVELS

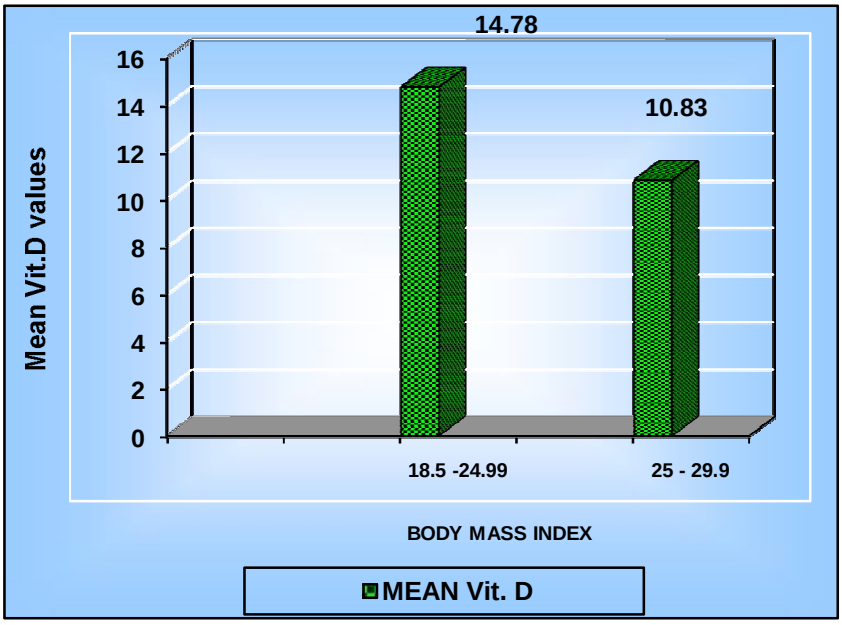


TABLE B5 : GRAVIDA & VITAMIN D LEVELS

Gravida	Vit. D (ng/ml)	
	Mean	S.D.
Primi	10.16	8.6
Multi	13.54	6.88
'p'	0.1295 Not Significant	

Primigravida and multigravida do not show any significant variation in concentrations of vitamin D in GDM group

Figure :16 GRAVIDA & VITAMIN D

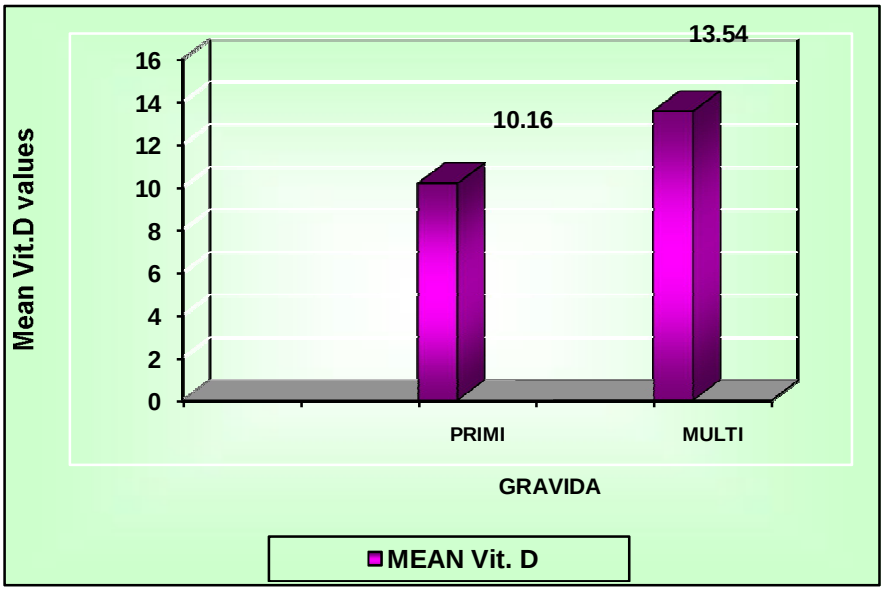


TABLE B6 : VITAMIN D STATUS AND OGTT IN GDM GROUP

VITAMIN D STATUS (ng/ml)	OGTT	
	Mean	S.D.
Severely deficient (< 5)	195.9	31.2
Moderate deficient (5.1 – 10)	194.3	31.4
Mild deficient (10.1 - 20)	175.4	20.2
Insufficient (20.1 – 30)	150.4	4.4
'p' Value	**0.0042 Significant**	

In GDM group, women with a mean high blood sugar level (195.9 ± 31.2 mg/dl) had 25-hydroxyvitaminD levels less than 5ng/ml. This showed that severe VDD was found in women with higher blood sugar levels .

Figure : 17 VITAMIN D STATUS AND OGTT LEVELS

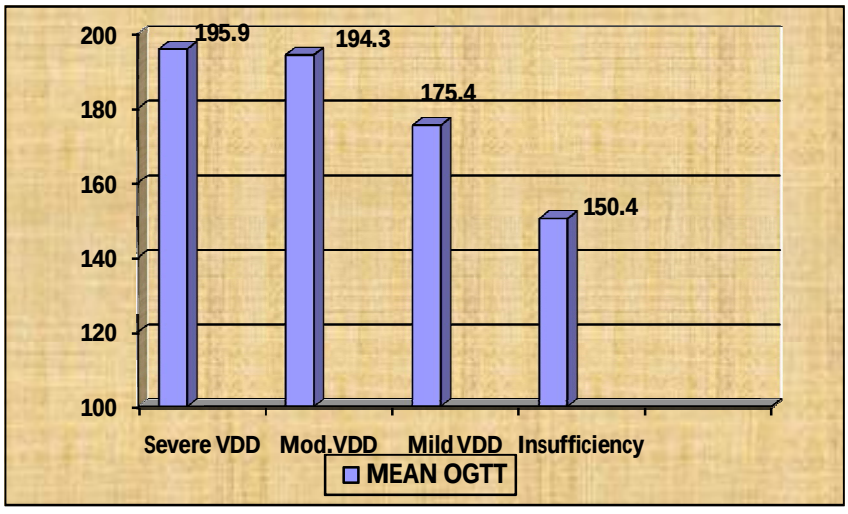
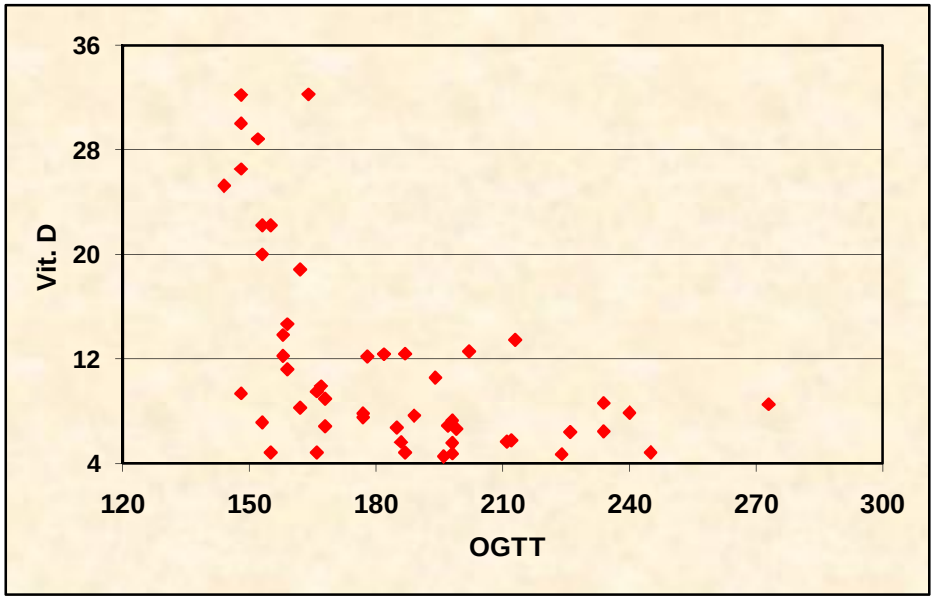


Figure 18 : CORRELATION BETWEEN OGTT AND VITAMIN D



Correlation coefficient between blood sugar levels by OGTT and vitaminD is found - 0.5455 (negative correlation). This shows that, as the vitamin D level decreases, the blood sugar level increases in women with GDM.

DISCUSSION

DISCUSSION

Pregnancy is a diabetogenic state since insulin requirements during pregnancy are increased[3]. The most important reason why pregnancy increases the diabetic tendency of asymptomatic women is the progressive increase in insulin resistance[18]. The glucose intolerance which occurs in pregnancy is GDM. GDM contributes to poor maternal and fetal complications in pregnancy as well as leads to future health risks like metabolic syndrome, obesity, and diabetes for the mother and fetus[1,19]

During the second half of pregnancy, there is increased synthesis of placental hormones which are responsible for insulin insensitivity as well as diabetogenic tendency[19]. There is also production of enzymes like placental insulinase by the placenta that increase the degradation of insulin[3]. The hyperglycemic state of pregnancy contributes a constant delivery of glucose to the growing fetus[1]. As a result the metabolic changes that occur under the influence of insulin and the anti-insulin hormones facilitate anabolism during feeding and catabolism during fasting[19].

Many studies emphasis the need of vitamin D in regulating glucose metabolism. Recent evidence suggest that vitaminD is essential for the release of insulin directly and indirectly[5].

In the present study , 50 women with GDM were taken as GDM group and 50 women with normal pregnancy who were age and BMI matched, as control group. The study was done between 24 to 28 weeks of pregnancy, as the diabetogenic tendency is maximum after second trimester of pregnancy due to the peaking of placental hormones which is done similar to the studies by Soheilykhah et al[16]., Maghbooli et al[17]., and Heather H Burris et al[50]., Wang O et al [55].,

The current study inferred that the mean concentration of vitamin D was statistically lower in GDM group as compared to controls. The mean 25-hydroxyvitaminD concentration in the GDM group was 11.78 ±7.86 ng/ml. The mean vitamin D level in control group was 31.48 ± 5.01 ng/ml. In GDM group, an increased occurrence of VDD was observed in this study. These findings were consistent with the results of Soheilykhah et al[16], Maghbooli Z et al[17], Burris HH et al[50], Cho GJ et al [46], Nishad AH et al., [69], Aghajafari F et al[70] ., Napartivaumnuay N et al [51]., and Wei SQ et al [71].

Soheilykhah et al., also concluded that women with VDD had 2.66 fold higher possibility of GDM when compared to control group[16]. Cuilin Zhang C et al., observed that, for every 5ng/ml reduction in serum 25-hydroxyvitaminD level in the serum, there was 1.29 times increased risk of Gestational diabetes[47].

Madhu Jain et al., found that there was an higher frequency of VDD in early pregnancy. The GDM group showed lower 25-hydroxyvitaminD levels (11.93 ± 3.42 ng/ml) compared to normal pregnancy (22.26 ± 15.28 ng/ml) with $p < 0.0001$ and found significant[21]. This finding is consistent with the current study.

The observation of Wang O et al., was that low levels of maternal serum 25-hydroxyvitaminD were linked to insulin resistance as well as development of GDM[55]. The same findings were reported by Wei SQ et al in a meta-analysis of 24 studies[71].

In current study , 84% of women with GDM showed VDD, in which 14% of women in GDM group were severely vitamin D deficient (<5ng/ml), 46 % of women with GDM reported moderate deficiency (5.1 to 20ng/ml), 24% of GDM women showed mild deficiency (10.1 to 20ng/ml), 10% showed insufficiency (20.1 -30 ng/ml) and 6% had normal vitamin D levels (>30ng/ml) .

Women with normal OGTT showed 4% VDD, 24% had insufficiency and 72% had normal levels of vit D. These findings were consistent with results of Soheilykhah et al[16]., Cho GJ et al[46].

Cho GJ et al [46]., inferred that Serum 25-hydroxyvitaminD concentrations were found significantly decreased in GDM compared to the normal pregnancy ($p < 0.01$) and 85% of GDM cases showed VDD (Vit D less than 20ng/ml).

It was evident from the current study that the mean OGTT level was 195.9 $\pm$ 31.2 mg% in severely vitaminD deficient women, 194.3±31.4 mg% in moderately deficient, 175.4 ± 20.2 mg% in mildly deficient and 150.4 $\pm$ 4.4 mg/% in women with insufficient vitaminD levels. It is inferred that vitaminD levels were inversely associated with the blood glucose levels. These findings were consistent with the results of Soheilykhah et al[16], Clifton- Bligh et al[72] ., Poel YHM et al [49] ., Ahmed El Lithy et al[48]., Madhu Jain et al[21]., Sue Lynn Lau et al[73].

Nishad AH et al., concluded that a positive correlation exists between 25- hydroxyvitaminD levels and insulin sensitivity[69] . The study also stated that VDD was more prevalent in women with GDM[69].

Aghajafari F et al ., reviewed 31 eligible studies and found that vitaminD insufficiency had an increased likelihood for developing GDM[70]. Sue Lynn Lau and coworkers observed that decreased concentrations of maternal 25-(OH)D were independently linked with poor glycemic control[73].

In the current study, the correlation coefficient between blood sugar levels obtained by OGTT and levels of vitamin D was found to be -0.5455 (negative correlation). This suggested that, lower the vitaminD levels, higher was the blood sugar levels . These results were consistent with the studies performed by Maghbooli et al [17]., Ahmed El Lithy et al[48] ., Poel YHM et al [49]., Burris HH et al[50]., Clifton Bligh et al[72]., Lau SL et al[73].

Maghbooli et al., in their study stated that the concentrations of 25-hydroxyvitaminD positively correlated with insulin sensitivity and VDD contributes to insulin insensitivity in pregnancy and is more prevalent in GDM than normoglycemic subjects [17].

Ahmed El Lithy et al., observed a significant inverse correlation between levels of vitaminD and HbA1c and as vitaminD level increases, the HbA1c levels tend to decrease, proving the influence of vitamin D in glycemic control[48]. Serum 25-hydroxyvitaminD concentration negatively correlates with the blood glucose levels after 1 hour of 50gm glucose challenge test ($p < 0.01$) as inferred by Burris HH et al[50].

A meta analysis which included 7 studies by Poel YHM et al., reported that 25-hydroxyvitaminD levels inversely correlated with GDM[49] which is consistent with the present study. Clifton- Bligh et al., showed an inverse correlation between 25-hydroxyvitaminD levels and FBS levels[72]. Rudnicki and Molsted-Pederson[15], demonstrated that women diagnosed having GDM, had a transient decrease in FBS levels after intravenous injection of calcitriol which proved that vitamin D may directly increase glucose absorption in the cells by increasing insulin sensitivity[15] and thereby the role of vitamin D in glucose homeostasis.

The study by Aanchal Sablok et al., states that lesser the 25-hydroxyvitaminD concentrations, higher is the possibility of GDM. Further, vitaminD supplementation had reduced such risks[56]. The same findings were reported by Lewis S et al.,[74] Jitendra Ingole and Sonali Ingole[75].

Zuhur SS and co-workers found that the maternal vitaminD concentrations were appreciably lower in GDM cases and also the risk of GDM was found to be higher with severe VDD[45]. Parlea et al., noted a higher incidence of GDM subsequently, in women having decreased 25-hydroxyvitaminD concentrations in early pregnancy which suggested that vitaminD might influence glucose tolerance[76].

ROLE OF VITAMIN D IN GLUCOSE HOMEOSTASIS

Vitamin D and β cell function/insulin secretion

Direct Actions

$1,25(OH)_2D_3$ + VDR

↓ RXR

VDR + RXR

↓

Vitamin D response elements (VDRE)

↓

Enhances transcriptional activation of insulin gene

↓

↑ Insulin Synthesis

Indirect Actions

1. Calcium flux through the β-cells and intracellular calcium Ca^+
2. Regulates calbindin
3. "Calcium Paradox"

Other Actions

Anti-apoptotic effect by

1. Modulating the generation and effects of cytokine
2. Down-regulating Fas-related pathways
3. Via calbindin by its ability to buffer intracellular calcium

Vitamin D and insulin sensitivity

Direct Actions

1. Stimulates expression of INS-R
2. Activation of PPAR – δ (transcription factor) – implicated in fatty acid metabolism in skeletal muscles and adipose tissues

Indirect Actions

1. Regulating extracellular calcium flux though the cells
2. Anti-apoptotic effect (by modulating through interaction of nuclear K-β (NF-κβ) and effects on cytokines
3. Through RAAS

All these findings inferred that vitamin D has an important influence on glucose homeostasis through calcium regulation. The probable mechanisms by which it influences glucose homeostasis and its deficiency leads to risk of GDM are :

- VitaminD (mainly calcitriol) acts by modulating pancreatic β-cell action as well as secretion of insulin by binding to VDR present on beta cells and thereby regulating the extracellular as well as intracellular calcium pools[22] .
- VitaminD acts directly on beta-cells to induce secretion of insulin via increasing calcium levels intracellularly through voltage sensitive calcium channels[14,77] and vit D supplementation improves insulin release[77.]
- Facilitates the stimulation of endopeptidases in the beta cells which are also calcium dependant and thereby helps to convert proinsulin to insulin[14].
- Vit D is important for maintaining the extracellular calcium so that to ensure intracellular calcium influx normally through the cell membranes[14,77]. This action helps in mediating depolarization induced release of insulin.
- It directly stimulates the transcriptional activity of insulin and its receptor genes[77] thereby enhancing secretion of insulin and its action on target tissues.

- It enhances expression of insulin receptors on target tissues. Causes upregulation of IRS-1 in muscles thereby improving insulin sensitivity[42].
- Vit D facilitates the action of glucose transporters . It mainly helps in recruitment of GLUT-4 to cell surface that improves glucose uptake[43].
- VitaminD enhances the expression of calbindin which protects β-cells from cytokine stimulated cell death[5] . Calbindin also modulates the insulin release by controlling calcium concentration within the cell[78].
- Decreased vitamin D levels lead to secondary increase in PTH which elevates calcium within the β -cells paradoxically , thereby impairing calcium signaling for insulin production - Calcium paradox[78].
- It acts a potent immune modulator which down regulates the transcription of pro-inflammatory mediators like interleukins which are responsible for autoimmune beta cell damage[5].
- It also stimulates the anti-apoptotic proteins (A20), that prevents β-cell apoptosis via down regulation of Fas-pathway[78].
- Increased expression of genes like PPAR γ , PGC1α concerned with glucose metabolism and insulin sensitivity[41].
- Vit D inactivates nuclear factor κB which affects β -cell existence and survival[78].

- Maintains the normal function of enzymes like hexokinase, glucose -6-phosphatase[44].
- Vit D inhibits the RAAS pathway and decreases the synthesis of angiotensin-II which inhibits insulin action[78].

RELATIONSHIP BETWEEN 25-HYDROXYVITAMIN D AND OTHER VARIABLES

In the current study, vitaminD level was correlated with different parameters like age, BMI, diet, residence, and gravida among the GDM group.

AGE AND VITAMIN D LEVELS

The mean level of vitamin D between 20 to 25 years of age was 15.98 $\pm$ 9.21ng/ml , between 26 -30 years was 9.97 $\pm$ 6.17 ng/ml, and between 31 -35 years was 6.6 $\pm$ 1.84 ng/ml . The p value obtained was 0.0054 and found to be significant. This inferred that as the age increases the vitamin D levels tend to decline[79]. The probable cause could be, as the age increases , the levels of 7-dehydrocholesterol decreases in the layers of skin and thus decreases vitaminD synthesis [80]. As vitamin D levels decrease, there is an increased risk of GDM.

BMI AND VITAMIN D LEVELS

In the present study, women with normal BMI and overweight were included . As obesity itself is found to increase the chance of developing GDM[81], obese women were excluded from the study. The mean BMI in GDM group was 26.23 ± 2.12 kg/m^2. The mean total 25-hydroxyvitaminD level was 14.78 ± 5.22 kg/m^2 in women with normal BMI and 10.83 ± 8.35 kg/m^2 in women with overweight in GDM group. The p value was 0.1305 and not significant which showed no statistical association between BMI and 25-hydroxyvitaminD concentrations.

RESIDENCE AND VITAMIN D LEVELS

A study conducted by Sahu et al[82]., reported a higher prevalence of VDD among pregnant women of rural region. But in another study done by Madhu Jain et al., VDD was highly prevalent in urban women than the rural women[21]. In the current study, the mean total 25-hydroxyvitaminD concentration in rural population was 11.01 ± 5.36ng/ml, and in urban population 12.03 ± 8.54 ng/ml . The p value obtained was 0.7009 and not significant .

It is inferred that there existed no significant link between vitaminD levels and residence. The probable cause of VDD may be due to increased urbanisation, use of sunscreens, greater pollution that decreases the exposure of women to sunlight, and poor outdoor activity.

DIET HABITS AND VITAMIN D LEVELS

The vegeterians constituted 54 % and non-vegeterians were 46% in the GDM group . The mean 25-hydroxyvitaminD level in vegeterians was 13.23±9.09ng/ml, and in non- vegeterians, it was 10.55 ± 6.54 ng/ml in the present study . The p value obtained was 0.2338 and not significant. So, the diet habits showed no significant link with the vitaminD levels in current study. The probable cause may be, vitamin D synthesis is largely influenced by sun exposure[83].

GRAVIDA AND VITAMIN D

The primigravida constituted 52% and multi gravida constituited 48% in GDM group . The mean vitamin D level in primi gravida was 10.16±8.6ng/ml and in multi gravida was 13.54 ± 6.88 ng/ml . The p value was 0.1295 and not significant which inferred that no link existed between vitamin D concentrations and gravida.

The current study inferred that vitaminD significantly influences glucose homeostasis, as well as its deficiency may lead to the development of GDM. Further, there existed no link between 25-hydroxyvitaminD levels and other parameters like BMI, diet, residence and gravida in the study. But as age increases, the vitamin D level decreases which may further increase the risk of GDM.

SUMMARY

SUMMARY

- Vitamin D status in GDM was assessed - Women in GDM group had lower vitaminD levels (Vit D deficient state)
- The mean serum total 25-hydroxyvitaminD level in GDM group was estimated and was 11.78 $\pm$ 7.86 ng/ml.
- The mean serum total 25-hydroxyvitaminD concentration in control group (women with normal pregnancy) was estimated and was 31.48 ± 5.01ng/ml .
- The 25-hydroxyvitaminD levels in GDM group were statistically lower as compared to control group. ($p<0.0001$)
- Correlation coefficient between blood glucose levels and vitamin D was -0.5455 (negative correlation). Vitamin D concentrations were negatively correlated with blood sugar levels in women with GDM, which inferred, as vitamin D level decreases, the blood sugar level increases.

CONCLUSION

CONCLUSION

In a developing country like India , Diabetes mellitus stands first among the fastest growing chronic diseases. Gestational diabetes contributes to the occurrence of diabetes mellitus in later life . So, identification of GDM is a major health concern in the society. There is an urgent need to discover and implement prophylactic measures to prevent this growing wave of diabetes.

Vitamin D deficiency is found to be an environmental and genetic factor that increases the risk of GDM recently. Due to urbanisation, lack of outdoor activities and spending most of the time in closed air conditioned rooms has led to increased incidence of VDD.

From the current study, it is found that vitamin D levels were found to be lower and deficient in women with GDM compared to women with normal pregnancy. This study throws light on the link between the deficiency of vitamin D and GDM. Also there is a negative correlation between the blood sugar levels and 25-hydroxyvitaminD concentrations which infer that, as vitamin D levels decrease , the blood sugar levels increase.

Vitamin D deficiency is considered as a preventable and modifiable risk factor for GDM. As GDM has adverse health impacts on the mother and fetus, screening for VDD in pregnancy is essential. Supplementation of vitamin D , encouraging outdoor activities and exercise which increases exposure to sunlight, may play a pivotal role in decreasing the incidence of GDM and diabetes mellitus in future .

LIMITATIONS

- Large sample size and follow up studies would be of great value.

- Follow up studies by estimating vitamin D levels from early pregnancy to delivery would provide a clear evidence to determine the link between VDD and GDM.

- Further, studies with vitamin D substitution and glycemic control in GDM women would strongly help in revealing influence of vitamin D in glucose homeostasis.

FUTURE SCOPE

The current study could be of public health importance as prenatal screening for VDD in the reproductive age group and proper supplementation of vitamin D may prevent maternal complications during pregnancy like gestational diabetes mellitus, preeclampsia and also fetal complications like rickets, diabetes, and bronchial asthma.

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ANNEXURES

CONSENT FORM

It has come to my knowledge that Dr.U.Kalpana Rani, Post Graduate student in the Department of Physiology, Coimbatore Medical College, Coimbatore is doing a research project on "**Assessment of Maternal Vitamin D status in Gestational Diabetes Mellitus**" as part of her dissertation. The procedure for collecting blood for estimation of vitamin D was explained to me clearly.

I hereby give my consent to participate in this study. The data obtained herein may be used for research and publication.

Name :

Place :

Date :

Signature :

ஒப்புதல் படிவம்

பெயர்_____வயது____,முகவரி_____

_____ஆகிய நான் உடலியங்கியல் துறை, கோவை மருத்துவ கல்லூரி பட்டமேற்படிப்பு மாணவி ஆகிய மரு.உ. **கல்பனா ராணி** அவர்கள் **''கர்ப்பகால சர்க்கரை நோய் உள்ள தாய்மார்களின் வைட்டமின் 'டி' நிலை பற்றிய ஆய்வு''** என்ற தலைப்பில் செய்யும் ஆய்வில் கலந்துக் கொண்டு ஒத்துழைக்க சம்மதிக்கிறேன்.

இந்த ஆய்வின் செய்முறை மற்றும் இது தொடர்பான அனைத்து விளக்கங்களையும் கேட்டுக்கொண்டு எனது சந்தேகங்களையும் தெளிவு படுத்திக்கொண்டேன் என்பதையும் தெரிவித்துக் கொள்கிறேன்.

நான் இந்த ஆய்வில் முழுமனதாக சுயசிந்தனையுடன் கலந்துகொள்வதுடன் எந்த நேரத்திலும் இந்த ஆய்விலிருந்து விலகிட எனக்கு உரிமை உண்டு என்பதையும் அறிவேன்.

இந்த ஆய்வில் எனது விவரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில் ஆட்சேபனை இல்லை/ விருப்பம் இல்லை என்பதை தெரிவித்துக்கொள்கிறேன்.

பெயர் :

இடம் :

தேதி :

கையொப்பம் :

PROFORMA

Name:

Age :

Occupation:

Address:

Gravida:

LMP:

EDD: Mobile No:

Duration of pregnancy:

Treatment:

Past history of HT/DM:

Family history of DM:

Previous history of GDM:

Taking any drugs:

General Examination:

Height:

Weight: BMI:

Blood pressure:

Pulse Rate:

Respiratory Rate:

Anemia :

Pedal edema :

System Examinations:

Cardiovascular System:

Heart sounds :

Respiratory System:

Breath sounds :

Central Nervous System:

Obstetric Examination:

P/A : size of uterus -

Fetal heart sound -

Investigations Done Already:

75 gm OGTT:

Haemoglobin:

USG Report:

MASTER CHART

WOMEN WITH GESTATIONAL DIABETES MELLITUS - GDM GROUP

NAME	GROUP	AGE	HT	WT	BMI	VIT. D	OGTT	GRAVIDA	WEEKS	FAMILY H/O	RESIDENCE	DIET
1	GDM	34	154	62.5	26.37	4.86	166	p	24	yes	R	V
2	GDM	33	156	64	26.22	8.26	162	m	24	no	U	NV
3	GDM	30	156	66	27.04	7.18	153	p	25	no	U	V
4	GDM	34	158	64	25.64	9.36	148	p	24	no	U	NV
5	GDM	24	154	62	25.31	9.54	166	p	26	no	R	V
6	GDM	26	155	54	22.5	12.58	202	m	26	no	U	V
7	GDM	27	149	56	25.45	7.91	240	m	24	yes	R	V
8	GDM	28	151	59	25.87	5.61	198	m	26	no	U	V
9	GDM	26	155	65	27.08	4.56	196	p	27	no	R	V
10	GDM	28	158	68	27.3	5.67	186	p	24	no	U	NV
11	GDM	26	156	68	27.98	30.03	148	p	26	no	U	NV
12	GDM	22	159	59.5	23.54	13.83	158	p	28	yes	U	NV
13	GDM	23	154	62.5	26.37	25.26	144	p	26	yes	U	V
14	GDM	24	157	68	27.64	32.26	164	m	24	no	U	NV
15	GDM	25	154	61	25.72	12.2	178	p	24	no	R	V
16	GDM	26	156	66	27.16	4.72	224	m	24	no	U	NV
17	GDM	29	149	64	28.82	8.56	273	m	25	no	U	V
18	GDM	26	154	66.5	28.05	6.76	185	m	24	no	U	V
19	GDM	24	154	61	25.72	5.68	211	p	27	no	U	V
20	GDM	22	146	59	27.69	6.89	197	m	26	yes	R	NV
21	GDM	27	153	59.5	25.42	7.66	189	p	24	no	U	V
22	GDM	22	152	57	24.67	22.22	153	m	24	no	U	V
23	GDM	34	146	59	27.69	4.86	155	p	24	yes	U	NV
24	GDM	32	153	67	28.63	6.46	234	p	26	no	U	NV
25	GDM	32	154	65.5	27.63	7.55	177	m	26	yes	U	NV
26	GDM	31	156	66	27.16	4.86	187	m	25	no	U	V
27	GDM	29	148	62.5	28.53	8.66	234	p	25	no	R	V
28	GDM	28	148	64	29.22	5.78	212	m	24	yes	U	NV
29	GDM	25	146	61	28.63	4.76	198	p	24	no	U	NV
30	GDM	27	152	68	29.43	6.86	168	p	25	no	U	V
31	GDM	22	153	53	23.55	12.22	158	p	26	yes	U	NV

WOMEN WITH GESTATIONAL DIABETES MELLITUS - GDM GROUP

NAME	GROUP	AGE	HT	WT	BMI	VIT. D	OGTT	GRAVIDA	WEEKS	FAMILY H/O	RESIDENCE	DIET
32	GDM	25	154	52	21.94	26.56	148	p	25	no	U	NV
33	GDM	26	148	64	29.22	18.86	162	m	25	yes	R	NV
34	GDM	28	154	61	25.72	14.65	159	m	25	no	R	V
35	GDM	26	146	61	28.63	22.22	155	m	24	no	R	NV
36	GDM	22	156	65.5	26.95	32.22	148	m	27	yes	U	V
37	GDM	23	155	64.5	26.87	28.86	152	m	24	no	U	NV
38	GDM	24	157	60	24.34	11.22	159	p	24	no	R	V
39	GDM	26	143	55	26.9	6.44	226	p	25	yes	U	NV
40	GDM	25	152	63	27.39	7.32	198	p	25	no	U	NV
41	GDM	28	157	62	25.15	4.88	245	p	25	no	U	V
42	GDM	27	148	56.5	25.68	6.68	199	p	24	no	U	V
43	GDM	27	168	55	19.48	9.95	167	m	24	yes	U	V
44	GDM	23	158	59	23.69	12.41	187	m	24	no	U	V
45	GDM	24	156	55.5	22.83	20	153	m	24	no	U	NV
46	GDM	25	155	69.5	28.95	7.84	177	m	25	no	U	NV
47	GDM	27	159	68	26.98	8.96	168	p	24	no	U	V
48	GDM	28	158	56	24.9	10.56	194	m	25	no	R	V
49	GDM	25	168	68	24.09	12.38	182	p	25	no	U	V
50	GDM	27	152	55	23.8	13.46	213	m	25	no	U	NV

HT - HEIGHT
WT - WEIGHT
BMI - BODY MASS INDEX
FAMILY H/O - FAMILY HISTORY OF DM

OGTT - ORAL GLUCOSE TOLERANCE TEST
P - PRIMIGRAVIDA
M - MULTI GRAVIDA

U - URBAN
R - RURAL
V - VEGETERIAN
NV - NON VEGETERIAN

WOMEN WITH NORMAL PREGNANCY - CONTROL GROUP												
NAME	GROUP	AGE	HT	WT	BMI	VIT. D	OGTT	GRAVIDA	WEEKS	Family h/o	RESIDENCE	DIET
1	CONTROL	27	158	60	24.09	30.66	132	p	24	no	U	NV
2	CONTROL	23	154	58.5	24.7	24.44	124	p	24	no	U	V
3	CONTROL	25	149	56	25.22	31.33	130	p	24	no	U	V
4	CONTROL	22	152	59	25.54	26.62	96	p	25	yes	R	NV
5	CONTROL	22	156	55	22.63	28.56	112	m	25	no	U	V
6	CONTROL	24	156	56.5	23.25	36.52	126	p	25	no	U	NV
7	CONTROL	32	159	74	29.36	44.42	130	m	24	no	U	V
8	CONTROL	25	148	60	27.27	28.22	110	m	26	no	R	NV
9	CONTROL	26	155	58	24.16	35.54	122	m	26	yes	U	NV
10	CONTROL	23	151	56.5	24.78	23.37	102	m	26	no	U	V
11	CONTROL	24	152	68	29.56	29.87	86	m	25	no	U	V
12	CONTROL	25	156	72	29.62	31.15	99	p	25	no	U	V
13	CONTROL	28	162	58	22.13	32.23	106	p	24	yes	U	NV
14	CONTROL	33	153	60	25.64	26.6	111	p	24	no	R	V
15	CONTROL	30	161	52	20.07	27.76	122	p	24	no	U	NV
16	CONTROL	23	157	64	26.01	29.78	100	p	24	no	R	NV
17	CONTROL	24	149	64	29.09	34.28	128	m	24	no	U	NV
18	CONTROL	23	153	61	26.06	30.12	113	m	24	no	U	NV
19	CONTROL	23	151	53	23.24	32.22	136	m	25	no	U	V
20	CONTROL	21	159	65.5	25.99	31.44	123	m	26	yes	U	NV
21	CONTROL	20	153	68	29.05	30.04	105	p	25	no	R	NV
22	CONTROL	23	163	59	22.26	32.34	98	p	25	no	U	NV
23	CONTROL	21	159	63	25	17.42	88	p	26	no	U	V
24	CONTROL	22	151	62.5	27.41	30.46	109	p	25	no	U	V
25	CONTROL	25	150	66	29.33	36.23	116	p	24	no	U	V
26	CONTROL	21	152	59	25.54	30.22	129	p	24	no	U	V
27	CONTROL	21	159	55.5	22.02	30.56	131	m	24	yes	R	NV
28	CONTROL	26	155	59	24.58	31.11	120	m	25	no	U	NV
29	CONTROL	23	154	60.5	25.52	28.2	100	m	25	no	U	NV
30	CONTROL	22	158	56.5	22.69	38.2	108	p	25	no	R	V
31	CONTROL	22	155	50	20.83	30.12	88	m	24	yes	U	NV

WOMEN WITH NORMAL PREGNANCY - CONTROL GROUP												
NAME	GROUP	AGE	HT	WT	BMI	VIT. D	OGTT	GRAVIDA	WEEKS	Family h/o	RESIDENCE	DIET
32	CONTROL	21	149	52	23.42	32.32	127	m	24	no	U	V
33	CONTROL	29	145	54.5	25.95	34.44	134	m	24	no	U	NV
34	CONTROL	30	158	64	25.7	36.56	122	m	24	no	U	NV
35	CONTROL	30	153	69	29.48	28.42	110	p	25	no	U	V
36	CONTROL	29	151	62	27.19	18.24	106	m	24	yes	U	NV
37	CONTROL	27	149	59	26.57	42.28	134	m	25	no	R	NV
38	CONTROL	26	153	66	28.2	31.56	121	p	24	yes	R	V
39	CONTROL	22	147	63	29.16	32.77	111	m	24	no	U	V
40	CONTROL	31	156	60	24.69	40.26	104	m	25	yes	U	V
41	CONTROL	33	155	65.5	27.29	27.45	84	m	27	no	U	NV
42	CONTROL	32	157	72.5	29.47	32.78	103	p	27	no	U	NV
43	CONTROL	20	149	58.5	26.35	35.57	136	p	25	no	U	V
44	CONTROL	25	152	56	24.24	36.62	96	m	28	no	R	V
45	CONTROL	30	150	51	22.66	30.66	104	p	28	no	U	V
46	CONTROL	31	155	53	22.08	37.45	106	p	26	yes	U	V
47	CONTROL	23	153	49.5	21.15	31.24	121	p	26	no	R	NV
48	CONTROL	22	150	56	24.88	30.12	112	p	27	no	U	NV
49	CONTROL	26	154	69	29.11	30.96	124	p	26	no	U	V
50	CONTROL	29	150	55	24.44	34.34	102	p	27	yes	U	NV

HT - HEIGHT
WT - WEIGHT
BMI - BODY MASS INDEX
FAMILY H/O - FAMILY HISTORY OF DM

OGTT - ORAL GLUCOSE TOLERANCE TEST
P - PRIMIGRAVIDA
M - MULTI GRAVIDA

U - URBAN
R - RURAL
V - VEGETERIAN
NV - NON VEGETERIAN